RISK FACTORS FOR RETINAL VASCULAR CHANGES IN CHILDREN AND PREGNANT WOMEN

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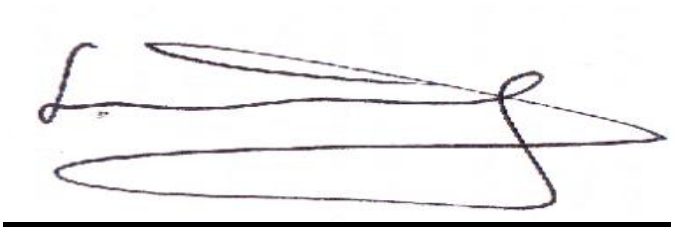
Declaration

I hereby declare that the thesis is my orginal work and it has been writeen by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in university previously.



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SUMMARY

Background:

The retinal blood vasculature is accessible to noninvasive visualization allowing for investigation of structural and pathologic features of the microcirculation and its relationship to systemic vascular diseases. Retinal vascular changes, such as retinal arteriolar narrowing and venular widening, have now been shown to be associated with various cardiovascular risk factors (e.g., hypertension, diabetes) and clinical cardiovascular disease (CVD) in the adult general population. However, such associations have not been investigated in young children and pregnant women, two specific populations with possible early predisposition of major systemic disease later in life. This thesis aims to describe retinal vascular changes and examine the major risk factors associated with such changes in Singapore children and pregnant women.

Methods:

Two studies were included in this thesis. First, a total of 586 healthy Singapore Chinese children aged 4 to 16 years were recruited in the Strabismus, Amblyopia and Refractive Error Study in Singaporean Chinese Preschoolers (STARS) from May 2006 through October 2008 and the STARS Family study from March 2008 through March 2010. Second, 824 healthy pregnant women aged 18–46 years were recruited as part of the Growing Up in Singapore Toward Health Outcomes (GUSTO) study. In both cohorts, retinal photography was performed and retinal vascular caliber was measured using a computer-based method.

Systemic factors such as BP, BMI, skinfold thickness, ocular biometric parameters and mental health assessments were measured according to standard protocols. Demographic information was collected at clinic interviews by trained staff. Retinal photographs were taken with a Canon 45° digital retinal camera and were subsequently graded by well-validated systems (IVAN, Interative Vessel Analysis; SIVA, Singapore I Vessel Assessment). Masked reliability tests had been performed before data on retinal vascular parameters were processed for statistical analysis. Multivariate regression analysis was used to explore the associations between a range of risk factors and retinal microvascular characteristics. Furthermore, the potential predictive values of retinal microvasculature on incidence of gestational complications were studied.

Results:

All variables of interests were approximately normally distributed both in children and pregnant women. The mean central retinal artery equivalent (CRAE) and vein equivalent (CRVE) were 155.34 μm and 218.80 μm in boys and 161.07 μm and 224.17 μm in girls, respectively. After adjusting for major potential confounders, each 10 mm Hg increase in systolic BP was associated with a 2.00 μm narrowing in retinal arterioles and a 2.51 μm widening in retinal venules. Each standard deviation (SD) increase in triceps skinfold thickness (TSF) (4.49 mm) and BMI (3.52 kg/m^2) was associated with a 2.94 μm and a 3.40 μm widening in retinal venular calibre, respectively. Each 1.0mm increase in axial length was associated with a 3.52μm decrease and a 5.55μm decrease in retinal arteriolar caliber and retinal venular caliber, respectively. By using specific cut-offs to classify childhood hypertension and

childhood overweight/obesity, children with BP above thresholds tended to have narrower retinal arteriolar caliber and children with BMI/TSF above thresholds tended to have wider retinal venular caliber, compared with those with BP, BMI or TSF below thresholds.

The mean CRAE and CRVE were 120.09 μm and 169.94 μm in Chinese pregnant women, 122.59 μm and 172.81 μm in Malay pregnant women and 122.44 μm and 169.21 μm in Indian pregnant women, respectively. In multivariate analysis, every 10 mmHg increase in mean arterial blood pressure (MABP) was associated with a 1.9 μm reduction in retinal arteriolar caliber, a 0.9° reduction in retinal arteriolar branching angle, and a 0.07 reduction in retinal arteriolar fractal dimension, respectively. Patients classified into high-risk group in developing pre-eclampsia (MABP≥90 mmHg) was twice as likely (Odds Ratio: 2.1, 95% CI: 1.0, 4.4) to have generalized retinal arteriolar narrowing compared with those classified into low-risk group (MABP<90 mmHg). Compared with mothers with normal weight, obese mothers (pre-pregnancy BMI>30.0 kg/m^2) had narrower retinal arteriolar caliber (118.81 vs. 123.38 μm, $p<0.001$), wider retinal venular caliber (175.81 vs. 173.01 μm; $p<0.01$) and increased retinal venular tortuosity (129.92 vs. 121.49 $x10^{-6}$; $p<0.01$). Each SD increase in the Edinburgh Postnatal Depression Scale (EPDS) (4.49 scores) and in the Pittsburgh Sleep Quality Index (PSQI) (2.90 scores) was associated with a 0.80μm ($p=0.03$) and a 1.22μm ($p=0.01$) widening in retinal arteriolar caliber, respectively. However, changes in retinal vascular parameters were not associated with incidence of gestational complications reported upon delivery.

Conclusion:

In both children and pregnanet women, elevated blood pressure and greater BMI were significantly associated with retinal arteriolar narrowing and/or retinal venular widening. Longer axial length and greater corneal curvature were associated with retinal arteriolar and venular narrowing in children. Negative antenatal emotions such as depressive symptoms and poor sleep quality were associated with retinal arteriolar widening in pregnant women.

Our study is the first study comprehensively investigating a range of systematic risk factors and retinal vascular changes, reflecting the systemic microcirculation *in vivo*, in young children and pregnant women. Our study provides evidence that hypertension and obesity in childhood and in mid-term pregnancy were associated with subclinical vascular changes, and thus further insights on the potential pathophysiological pathways and mechanisms involved in the development of major metabolic and cardiovascular diseases later in life.

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CHAPTER ONE

1. Literature Review

1.1 Introduction

The retinal microcirculation is part of the microcirculation *in vivo*, of which the vascular tree consisted of retinal arteriole and venules that contain neither internal elastic lamina nor a continuous muscular coat.[1] Based on the specific architectural anatomy, changes on retinal microvasculature can be reflected promptly and sensitively.

With advances in retinal photographic techniques, retinal vascular network can now be visualized and graded conveniently and non-invasively.[2, 3] Also, various semi-automated, computer-based retinal imaging programs have proven to be highly accurate and reproducible in assessing architectural changes in retinal vascular network *in vivo*.[4] A series of quantitative measurements of vascular characteristics such as retinal vascular caliber, retinal vascular tortuosity, retinal vascular branching angle and retinal vascular fractal dimension[5] have been suggested to represent microcirculation in vivo.

Furthermore, in the past 10 years, a number of multi-ethnic and population-based cohorts conducted in the general population (children with 7 years and above and adults) have suggested that early signs of changes in retinal vascular parameters not only were associated with systemic, environmental and genetic factors such as age,

blood pressure, body mass index (BMI), lower income, life style of smoking and alcohol dinking and ethnicity, but also carried predictive value for incidence of various medical conditions including vascular risks (such as hypertension and obesity)[5-11] and vascular diseases (such as kidney disease, cardiovascular diseases and stroke) for clinical and epidemiological research.[12-17] However, the range of established risk factors influencing variation in retinal microvasculature haven't been fully investigated in preschoolers (6 years and below) and pregnant women.

1.2 Measurements of Retinal Vascular Parameters

1.2.1 Retinal Vascular Caliber

Recent population-based studies have used computer-assisted programs to measure individual arterioles and venules and to combine them according to formulas developed firstly by Parr and Spear,[18, 19] subsequently modified by Hubbard *et al*,[4] and further improved by Knudtson *et al*.[20] The use of computer-assisted programs differs in all population-based epidemiological studies. For example, Computer Assisted Image Analysis of the Retina program (CAIAR) and Retinal Image MultiScale Analysis was used in UK adult studies,[21, 22] Retinal Imaging Software Fractal (IRIS-Fractal) was used in Australian children study,[23-26] Non-mydriatic Vessel Analyser (SVA-T) was used in German children study,[27] Interactive Vessel Analysis (IVAN) was widely used in US studies[28-30] and Asian studies[8, 31] while Singapore I Vessel Assessment (SIVA) was newly developed and applied in recent Singapore studies.[5, 11]

Regardless of the various models of computer-assisted programs, the calibration of all these programs works similarly. After determining the true size of the optic disk and locating retinal vessels with minimum detectable width varied by different computer-assisted programs, calibration of the computer-assisted program will generate three fundamental variables, which are projected caliber size of the central retinal arteriole equivalent (CRAE), the projected caliber size of central retinal venular equivalent (CRVE) and the ratio of the two variables (arteriole-to-venule ratio [AVR]). With the substantial reproducibility demonstrated in recent studies (intra- and inter-grader correlation coefficient ranged from 0.67-0.99), computed-assisted programs have been proved to be precise and reliable for assessing structural retinal vascular caliber changes among general population. The outputs of IVAN and SIVA computer programs are shown in **Figure 1** and **Figure 2**.

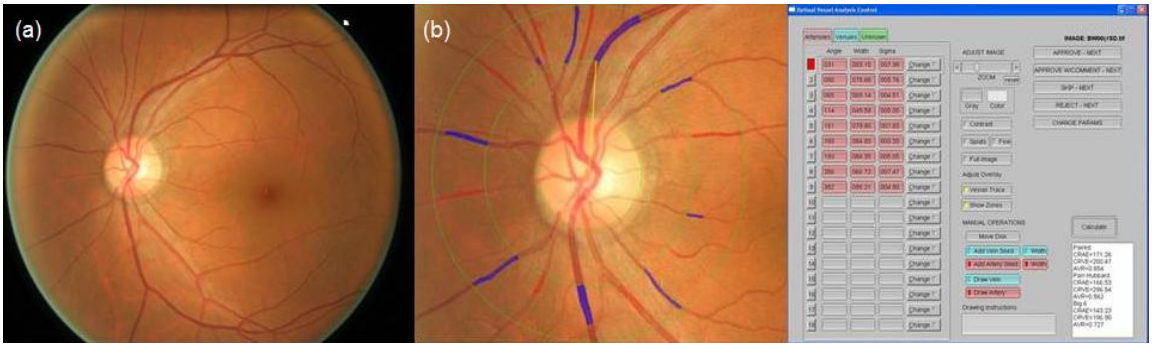


Figure 1. (a) Retina allows for a noninvasive visualization of human microcirculation. (b) Computer-assisted program for the measurement of retinal vascular caliber to quantify structural vascular microcirculatory changes. Zone B is marked in IVAN software by 0.5 to 1.0 optic disc diameter away from the margin of optic disc. The biggest eight retinal vascular arterioles and venules were located and assessed within zone B.

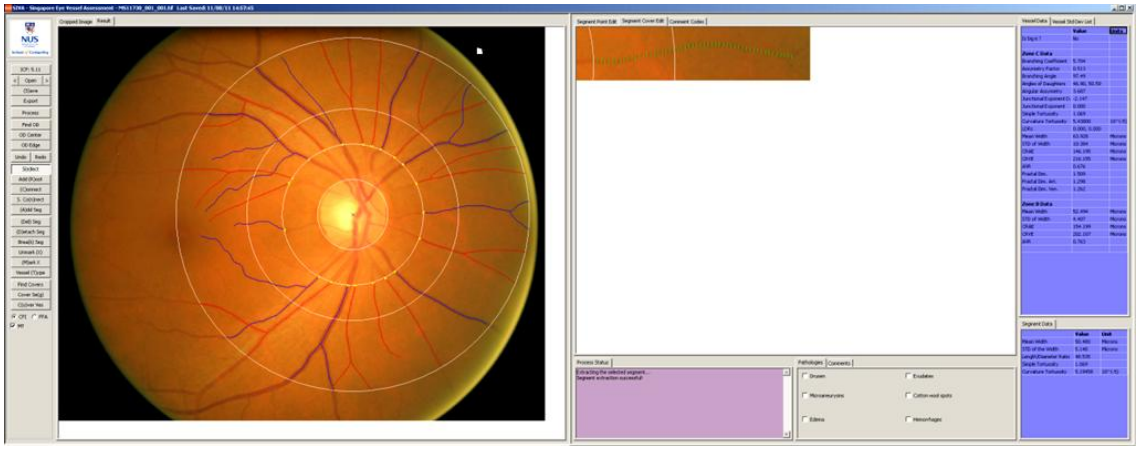


Figure 2. A screenshot of a computer-assisted program for measurement of new geometrical retinal vascular parameters from retinal fundus photograph. Zone B and zone C are marked in IVAN software by 0.5 to 1.0 and 0.5 to 2.0 optic disc diameter away from the margin of optic disc, respectively. All retinal arterioles and venules larger than 25 µm are marked and assessed within zone B and zone C.

1.2.2 Novel Retinal Vascular Parameters

With innovative technology and development in retinal photography, image processing, analysis and computer vision techniques, objective and quantitative assessment of novel classes of retinal vascular branching parameters were performed reliably and rapidly. The human circulatory system conforms to optimum design principal (Murray principle of minimum work).[32] Deviations or alterations from optimal architecture are speculated to result in impaired microcirculatory transport, reduced efficiency and thereby, a greater risk of clinical cardiovascular disease.[33, 34] Previous studies have focused on one retinal vascular parameter – caliber (diameter) of the retinal vessels, for quantifying the retinal vascular changes using the advent of computer-assisted methods. In addition to retinal vascular caliber (e.g. retinal

arteriolar narrowing), newer studies are now starting to examine the branching pattern of the retinal vascular tree, which may capture the "optimal state" of the retinal microcirculation and provide additional and independent cardiovascular risk information to enable better predictive ability of cardiovascular outcomes.

The novel geometrical retinal vascular parameters are:

- **Retinal vascular tortuosity**: is derived from the integral of the curvature square along the path of the vessel, normalized by the total path length, which takes into account the bowing and points of inflection (**Figure 3a**).[11]

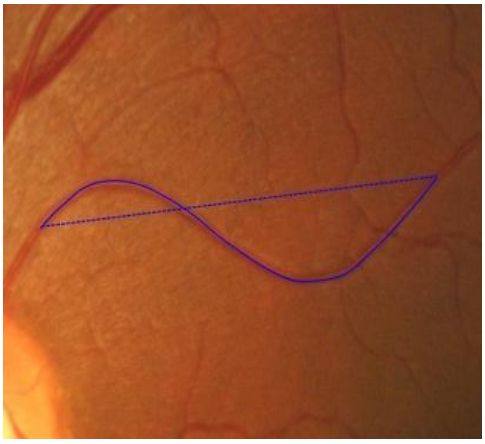


Figure 3A. Retinal vascular tortuosity.

- **Fractal dimension**: retinal vascular tree is a "self-similar" structure which can be summarized in terms of the fractal dimension quantifying the complexity of the whole branching pattern of the retinal vascular tree. Fractal dimension is defined as the gradient of logarithms of the number of boxes and the size of the boxes (**Figure 3b**).[35]

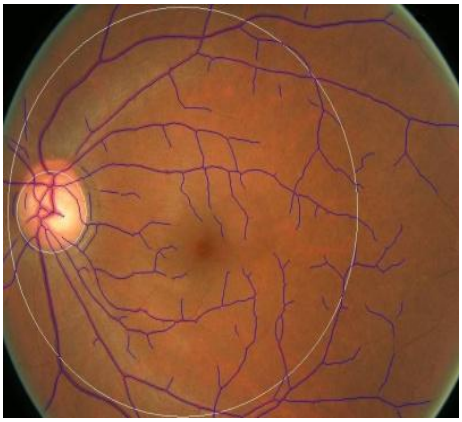


Figure 3B. Retinal vascular fractal dimension.

- **Branching angle**: is defined as the first angle subtended between two daughter vessels at each bifurcation (**Figure 3c**).[36]

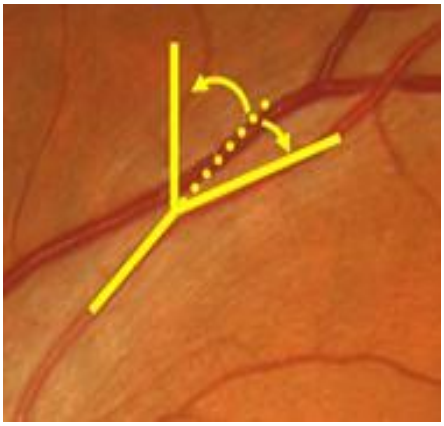
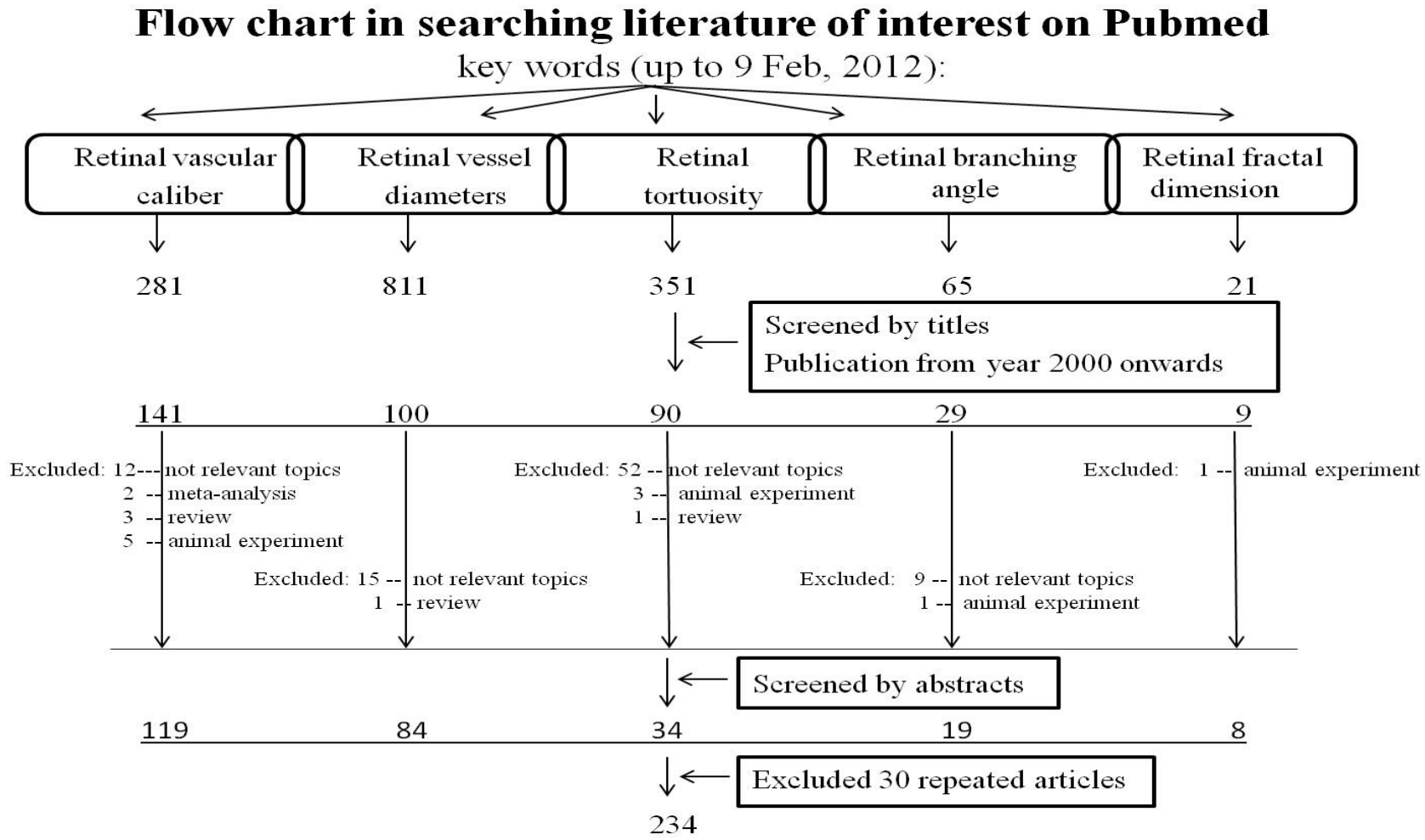


Figure 3C. Retinal vascular branching angle.

1.3 Risk factors for Retinal Vascular Caliber Changes in General Population

A systematic MEDLINE search on National Institute of Health's PubMed (website: www.ncbi.nlm.nih.gov/pubmed, 10 February 2012) was conducted initially using the following keywords: Retinal vascular caliber (281), retinal vessel diameters (811), retinal tortuosity (351), retinal fractal dimension (65) and retinal branching angle (21). Relevant abstracts published from 2000 onwards, accessible computer links to these abstracts and a preliminary list of possible articles from this search were compiled. There were 284 original articles in total included in our systematic review.

Flow chart to illustrate the searching and selection of literatures:



However, non-MEDLINE-based manuscripts, books and book chapters, unpublished data and ongoing research were not included. In this literature review, only the best 8-10 citations (such as evaluated by sample size, study power, study design, epidemiological methodology etc.) are listed in each category of risk factors.

1.3.1 Systemic Factors with Retinal Microvasculature

Growing evidence has shown that variations in retinal vascular parameters are associated with a range of systemic conditions.

1.3.1.1 Associations with Demographic Status (Age, Gender and Ethnicity)

Age increment was associated with retinal arteriolar and venular narrowing among people 30 years and above.[7-9, 29, 37-39] Each decade increase in age was significantly associated with 1.71-4.2 µm and/or 1.42-3.9 µm narrowing in retinal arteriolar caliber and retinal venular caliber, respectively across Beaver Dam Eye Study, Blue Mountains Eye Study, Wisconsin Epidemiological Study of Diabetic Retinopathy, Japanese Funagata Study and Israeli clinical study in multi-adjustments (**Table 1**).[7, 8, 29, 37-39] The Multi-Ethnic Study of Atherosclerosis (MESA) reported narrowing trends in retinal arteriolar caliber (147.1 vs. 139.8 µm, $p<0.001$) and retinal venular caliber (217.3 vs. 207.6 µm, $p<0.001$) with participants aging from 45 years to 84 years.[9] There were no adjustments in multivariate models.

Furthermore, a few recent studies have reported relationships between increased age and decreased novel retinal vascular parameters.[5, 40-42] In Singapore Malay Eye Study (SiMES), participants aged 40-49 years vs. those aged 70-80 years had smaller retinal arteriolar tortuosity (3.45 vs. 2.71×10^4, p trend<0.001) and smaller retinal venular tortuosity (4.97 vs. 4.31×10^4, p trend=0.001), respectively,[5] while in Sydney

Pediatric Diabetes Study Sasongko *et al*. each SD (1.5 years) increase in children and adolescents aged 12-20 years was associated with 0.73 $x10^3$ (p-0.035) and 0.90 $x10^3$ (p=0.002) decrease in retinal arteriolar and venular tortuosity, respectively.[42] Similarly, older people tended to have smaller retinal arteriolar and venular branching angle and smaller fractal dimension,[5] the latter of which was suggested by Doubal *et al.*[40] and Liew *et al*. (**Table 2**).[41]

However, there was a lack of evidence showing a relationship between gender and retinal vascular parameters in general population. In the Blue Mountains Eye Study, retinal arteriolar caliber and AVR were both higher in women than men,[39] while the Beaver Dam Eye Study didn't report the consistency in its findings. [43]

The relationship of ethnicity/races and retinal vascular parameters has been quite fundamentally studied, since some cardiovascular risk factors varied in different ethnic groups. For example, in MESA, black and Hispanic participants were more likely to have diabetes, obesity, hyperlipidemia and systemic inflammation than whites.[9] Therefore, the racial/ethnic differences in retinal vascular caliber may partly reflect variations in susceptibility of the retinal vasculature to cardiovascular risk factors or other processes like genetic factors which would be described in the next chapter.

In SCES, East Asian had a 11.1 μm wider retinal venular caliber than European caucasian.[44] Also, the darker the iris color (dark brown vs. Tan brown vs. Hazel green vs. blue), the wider the retinal arteriolar caliber (169.8 vs. 164.1 vs. 161.0 vs. 157.1 μm, p trend<0.0001) and the retinal venular caliber were found (237.7 vs. 228.9 vs. 223.6 vs. 218.4 μm, p trend<0.0001).[44] In MESA, Hispanic and Black participants

had a wider retinal arteriolar and venular caliber than Chinese and White participants.[9] Similarly in SCORM children, Cheung et al. found that there was an increasing trend in retinal venular caliber across Chinese children, Indian children and Malay children (p trend=0.02).[45] Mahal et al. studied 51 subjects with type 2 diabetes aged 40-65 years and found that African-Caribbean had wider retinal arteriolar caliber (82 vs. 76 µm, p=0.03) than European (**Table 3**).[21]

Therefore, the associations between ocular pigmentation and ethnicity and retinal vascular caliber may be a reflection of relationship between genetic variance and retinal vascular caliber.

Table 1. Associations between Age and Retinal Vascular Caliber

	Study	Study population and study design	Sample size and response rate	Age range	Age	Retinal arteriolar caliber	Retinal venular caliber
1 2012	Gepstein B Clinical study	Annual check-up patients Hospital-based, cross-sectional study	285 patients	33-87 yrs	each 10-yr ↑	1.71 μm ↓ (p=0.012)	1.42 μm ↓ (p=0.04)
2 2008	Sun C SiMES	Singapore Malays Population-based, cross-sectional study	3019 out of 3280 92.0%	40-80 yrs	each 10-yr ↑	n.s.	2.86 μm ↓ (p<0.001)
3 2006	Klein R WESDR	370 with diabetes diagnosed with 30+ years Population-based, cross-sectional study	1370 with type 2 DM	30+ yrs	each 10-yr ↑	2.0 μm ↓	2.5 μm ↓
4 2006	Wong TY MESA	Multi-ethnic Americans Population-based, cross-sectional study	5979	45-84 yrs	45-54 yr vs. 55-64 yr vs. 65-74 yr vs. 75-84 yr	147.1 (0.32) vs. 144.5 (0.34) vs. 142.6 (0.35) vs. 139.8 (0.55) μm p trend<0.001	217.3 (0.49) vs. 215.1 (0.53) vs. 212.3 (0.54) vs. 207.6 (0.90) μm p trend<0.001
5 2006	Kawasaki R Funagata study	Japanese Population-based cross-sectional study	1481	35+ yrs	each 10-yr ↑	2.4 μm ↓ (p<0.0001)	1.8 μm ↓ (p<0.0001)
6 2003	Wong TY BDES	American patients Population-based, cross-sectional study	4926	43-84 yrs	each 10-yr ↑	2.1 μm ↓ (95% CI: 1.5, 2.7)	n.s.
7 2003	Leung H BMES	Australian Caucasians Population-based, cross-sectional study	3654	49+ yrs	each 10-yr ↑	4.2 μm ↓ (p<0.001)	3.9 μm ↓ (p<0.001)

Abbreviations: SiMES: Singapore Malay Eye Study; WESDR: Wisconsin Epidemiological Study of Diabetic Retinopathy; MESA: Multi-Ethnic Study of Atherosclerosis; BDES: Beaver Dam Eye Study; BMES: Blue Mountains Eye Study.

Table 2. Associations between Age and Retinal Vascular Tortuosity, Branching Angle and Fractal Dimension

	Study	Study population and study design	Sample size and response rate	Age range	Age	Retinal arteriolar characteristics	Retinal venular characteristics
1 2011	Cheung C SiMES	Singapore Malays Population-based, cross-sectional study	2915 out of 3280 88.8%	40-80 yrs	40-49 yrs vs. 70-80 yrs	**Arteriolar tortuosity:** 3.45 vs. 2.71 $x10^4$ (p trend<0.001) **Arteriolar branching angle:** 79.15 vs. 72.46 (P trend<0.001) **Fractal dimension:** 1.425 vs. 1.382 (P trend <0.001)	**Venular tortuosity:** 4.97 vs. 4.31 $x10^4$ (p trend=0.001) **Venular branching angle:** 80.18 vs. 77.62 (p trend<0.001)
2 2010	Sasongko M Sydney Pediatric Diabetes Study	Sydney children and adolescents with type 1 diabetes Cross-sectional study	944 out of 1159 81.4%	12-20 yrs	each SD ↑ (SD=1.5 yr)	**Arteriolar tortuosity:** 0.73 $x10^3$ ↓ (-1.37, -0.10) (p=0.035)	**Venular tortuosity:** 0.90 $x10^3$ ↓ (-1.54, -0.26) (p=0.002)
3 2010	Doubal FN Clinical study	Hospital based, case-control study	166 out of 183 eligible 86 with lacunar stroke 80 with cortical stroke	Mean age 67.3 yrs	each 10-yrs↑	**Monofractal dimension (Dbox)**: 0.10 ↓ (p<0.001) **Multifractal dimension (DO):** 0.11 ↓ (p<0.001)	

	Study	Study population and study design	Sample size and response rate	Age range	Age	Retinal arteriolar characteristics	Retinal venular characteristics
4 2008	Liew G BMES	Population-based cohort study	300 random samples 100 HTN 100 DM 100 without HTN	49+ yrs	↑	**Fractal dimension**: ↓ (r=-0.42, p=0.001)	

Abbreviations: SiMES: Singapore Malay Eye Study; BMES: Blue Mountains Eye Study.

Table 3. Associations between Ethnicity and Retinal Vascular Parameters

	Study	Study population and study design	Sample size and response rate	Age range	Ethnicity	Retinal arteriolar characteristics	Retinal venular characteristics
1 2008	Rochtchina E SCES	Population-based, cross-sectional study	1740 children	6 years	Iris color: Blue vs. Hazel green vs. Tan brown vs. Dark brown Ethnicity: East Asian vs. European Caucasian	CRAE: 157.1 vs. 161.0 vs. 164.1 vs. 169.8 µm P trend<0.0001 n.s.	CRVE: 218.4 vs. 223.6 vs. 228.9 vs. 237.7 µm P trend<0.0001 CRVE: 240.0 µm vs. 228.9 µm P<0.05
2 2008	Mahal S Clinical study	Observational study in patients with type 2 DM	51 subjects (22 Europeans, 29 African-Caribbeans) with Type 2 DM	40-65 years	Ethnicity: European vs. African-Caribbeans	CRAE: 76 vs. 82µm ↑ (p=0.03)	n.s.
3 2006	Wong TY MESA	Americans Population-based cross-sectional study	5979	45-84 yrs	Ethnicity: Hispanic vs. Black vs. Chinese vs. White	CRAE: 145.9 vs. 145.2 vs. 142.8 vs. 142.7 µm (p<0.001)	CRVE: 217.4 vs. 221.8 vs. 215.0 vs. 206.6 µm (p<0.001)
4 2006	Cheung N SCORM	Singapore Chinese School-based, cross-sectional study	768	7-9 years	Ethnicity: Chinese vs. Indian vs.Malay	n.s.	CRVE: 223.5 vs. 227.5 vs. 240.5 µm (p=0.02)

Abbreviation: SCES: Sydney Children Eye Study; SCORM: the Singapore Cohort Study of the Risk Factors of Myopia.

1.3.1.2 Associations with Socio-Economic Status

Only a few studies also looked at the associations between socio-economic status, parental hypertension history and retinal vascular caliber (**Table 4**). Jensen et al. and Sun et al. suggested a relationship between household income and retinal vascular caliber both in MESA and SiMES studies.[8, 46] By using quantitative measurements and analysis, Sun et al. found that people with monthly income less than USD 2040 had a 1.23 μm wider retinal venular caliber than those whose monthly income is USD 2040 and above ($p<0.01$).[8] Similarly, Jensen found that people with the lowest annual income (USD<20,000) and people with lower educational level (less than high school degree) had a wider retinal venular caliber than people with highest annual income (USD≥50,000) (210.1 vs. 215.8 vs. 218.6 μm, $p<0.001$) and people with highest educational level (college graduate) (211.5 vs. 214.7 vs. 217.1 μm, $p<0.05$), respectively.[46]

Table 4. Associations between Socio-Economic Status and Retinal Vascular Caliber

	Study	Study population and study design	Sample size and response rate	Age range	Risk factors	Retinal Arteriolar Caliber	Retinal Venular Caliber
1 2009	Jensen RA MESA	Multi-ethnic Americans; Population-based, cross-sectional study	6147 out of 6814 90.2%	45-84 yrs	Annual income: < USD 20,000 vs. USD 20,000-49,000 vs. ≥ USD 50,000 . Education: <high school degree vs. high school to associates degree vs. College graduate	144.4 vs. 143.9 vs. 143.4 µm P<0.05 144.8 vs. 144.9 vs. 142.1 µm P<0.001	217.1 vs. 214.7 vs. 211.5 µm P<0.05 210.1 vs. 215.8 vs. 218.6 µm P<0.001
2 2008	Sun C SiMES	Singapore Malays Population-based, cross-sectional study	3019 out of 3280 92.0%	40-80 yrs	Monthly Income: < USD 2040 vs. ≥ USD 2040	1.23 µm ↑ P<0.001	n.s.

Abbreviations: HTN: hypertension; SCES: Sydney Childhood Eye Study; MESA: Multi-Ethnic Study of Atherosclerosis; SiMES: Singapore Malay Eye Study.

1.3.1.3 Associations with Life Style Factors (Smoking and Drinking)

The relationship between life style factors (cigarette smoking and alcohol drinking) and retinal vascular caliber were quite well studied by a few large population-based and cross-sectional studies. All these studies done on middle-to-old age subjects reported mostly consistent findings that current cigarette smoking or past smoking was related with both retinal arteriolar and venular widening (**Table 5**).[8, 9, 28, 37, 47, 48]

After comparing current cigarette smokers with non-cigarette smokers in SiMES, the Atherosclerosis Risk in Community (ARIC) and MESA studies, researchers reported a 2.89-4.31 μm ($p<0.001$) widening in retinal arteriolar caliber and a 4.00-11.36 μm ($p<0.01$) widening in retinal venular caliber, respectively.[8, 9, 28] Similarly, a wider mean retinal arteriolar caliber (196.4 vs. 188.3 μm, $p=0.02$) and a wider mean retinal venular caliber (223 vs. 208 μm, $p=0.002$ in Israelite study; 230.0 vs. 219.2 μm, $p<0.0001$ in BMES) were reported in one hospital-based Israelite study and one population-based Australian study (BMES), by comparing current or past smokers with nonsmokers (**Table 5**).[37, 47]

As for alcohol drinking, the relative changes in retinal vascular caliber varied in different studies. For instance, current alcohol drinking was suggested to be associated with retinal arteriolar narrowing in ARIC (1.2 μm, $p<0.05$) and MESA (2.31 μm, $p<0.001$) but not in BMES.[9, 28, 47] Furthermore, current alcohol drinking was suggested to be associated with retinal venular widening in ARIC (0.9 μm, $0<0.05$) and BMES yet not in MESA (-2.14 μm, $p<0.001$) (**Table 5**).[9, 28, 47] This may be due to differences in sample size and studied populations.

Table 5. Associations between Life Style and Retinal Vascular Caliber

	Study	Study population and study design	Sample size and response rate	Age range	Life style factors	Retinal arteriolar caliber	Retinal venular caliber
1 2012	Gepstein B Clinical study	Annual check-up patients Hospital-based, cross-sectional study	285 patients	33-87 yrs	Smoking: present vs. past vs. nonsmoker	n.s.	↑ 223 vs. 214 vs. 208 µm (p=0.002)
2 2008	Sun C SiMES	Singapore Malays Population-based, cross-sectional study	3019 out of 3280 92.0%	40-80 yrs	Smoking: Current/past vs. nonsmoker	2.89 µm ↑ (p<0.001)	4.00 µm ↑ (p<0.001)
3 2008	Liew G ARIC	White and African Americans Population-based, cross-sectional study	8794 out of 12887 68.2%	48-73 yrs	Smoking: current vs. nonsmoker	n.s.	5.7 µm ↑ (p<0.05)
					Alcohol Drinking: current vs. nondrinker	-1.2 µm ↓ (p<0.05)	0.9 µm ↑ (p<0.05)
4 2007	Kifley A BMES	Australian whites Population-based, cross-sectional study	Baseline: 3006 out of 3654 82.27%	49+ yrs	Smoking: current vs. nonsmoker	196.4 vs. 188.3 µm (p=0.02)	230.0 vs. 219.2 µm (p<0.0001)
					past vs. nonsmoker	n.s.	222.6 vs. 219.2 µm (p=0.004)
					Alcohol Drinking: heavy drinker vs. nondrinker	200.9 vs. 189.5 µm (p=0.02)	233.0 vs. 220.6 µm (p<0.0001)
5 2006	Klein R WESDR	Population-based, cross-sectional study	1370 with type 2 DM	30+ yrs	Smoking: current vs. nonsmoker	5.6 µm ↑ (p<0.05)	11.6 µm ↑ (p<0.05)

	Study	Study population and study design	Sample size and response rate	Age range	Life style factors	Retinal arteriolar caliber	Retinal venular caliber
6 2006	Wong TY MESA	American Population-based cross-sectional study	5979	45-84 yrs	Smoking: current vs. past/never Alcohol Drinking: current vs. past/never	4.31 μm ↑ (p<0.001) -2.31 μm ↓ (p<0.001)	11.36 μm ↑ (p<0.001) -2.14 μm↓ (p<0.001)

Abbreviations: SiMES: Singapore Malay Eye Study; ARIC, the Atherosclerosis Risk in Community; BMES, Blue Mountains Eye Study; WESDR, Wisconsin Epidemiological Study of Diabetic Retinopathy; MESA: Multi-Ethnic Study of Atherosclerosis.

1.3.1.4 Associations with Physical Activity and TV Viewing Time

With more and more studies establishing a strong relationship of metabolic syndromes and diseases with physical activity,[49-52] scholars have started to look for a possible pathophysiological mechanism by examining the association between physical exercises and TV viewing time and retinal microcirculation in the general population for the past five years. The findings were reported consistently across a few population-based studies in US, Singapore and Germany both in adults and children.[27, 53-58]

In Australian children with a mean age of 6.7 years, children in the highest tertile of outdoor sporting activity (≥0.71 hours/day) had a 2.2 μm wider retinal arteriolar caliber compared with the lowest tertile (<0.29 hours/day).[56] In German school children at 5th grade with a mean age of 11 years, physical activity was also associated with higher arteriolar-to-venule ratio (AVR) ($p=0.032$).[27] In MESA and ARIC American adults, participants classified into highest physical activity or intermediate level of work-related physical activity were found to have a 2.3 μm ($p<0.05$) or 1.0 μm ($p<0.05$) narrower retinal venular caliber compared with those classified into lowest physical activity or lowest work-related physical activity.[53, 58] Moreover, Hanssen et al. also found that runner with better fitness condition had a higher AVR ($p<0.01$), after studying 60 recreational runners in Germany, which was in accordance with the physical activity findings mentioned above.[57]

Since there is a strong correlation between lower outdoor physical activity and higher screen time or TV viewing time, researchers also found significant

relationships of higher levels in TV viewing time/Leisure-time/Screen time with retinal arteriolar narrowing and/or retinal venular caliber (**Table 6**).[53, 54, 56, 58] The magnitude of retinal vascular caliber changes was very similar to those changes observed in the physical activity analysis.

However, there has not been any study conducted on such relationships on retinal geometric parameters.

Table 6. Associations between Physical Activity, TV Viewing Time and Retinal Vascular Caliber

	Study	Study population and study design	Sample size and response rate	Age range	Physical factors	Retinal arteriolar caliber	Retinal venular caliber
1 2012	Hanssen H JuvenTUM 3 2008	Germany school children at 5th grade School-based cross-sectional study	578 out of 792 73.0%	Mean age 11.1 yrs	Physical inactivity:	AVR ↓ (p=0.032)	
2 2011	Anuradha S Singapore Prospective Study Program	Chinese, Indian and Malay adults	3866 out of 5157 75.0%	24-95 yrs	TV watching: **Female** >2 hours/day vs. ≤2 hours/day	1.28 µm↓ (-2.56, -0.03)	n.s.
					Leisure-time: **Male** 1st vs. 4th quartile	n.s.	1.51 µm↑ (0.01, 2.92)
3 2011	Gopinath B SCES	Sydney primary school pupils from year 1 to year 6 Multi-ethnicity Cross-sectional	1765 out of 2238 78.9%	mean age: 6.7 yrs	Outdoor sporting activities: 3rd (≥0.71 hrs/d) vs. 1st tertile (<0.29 hrs/d)	2.2 µm ↑ (p<0.05)	n.s.
					Screen time: 4th (≥2.36 hrs/d) vs. 1st (<1.07 hrs/d) quartile	2.5 µm ↓ (p=0.01)	n.s.
					TV watching time: 4th (≥1.5 hrs/d) vs. 1st (<0.57 hrs/d) quartile	2.3 µm ↓ (p=0.003)	n.s.
4 2011	Hanssen Longitudinal age-matched	Recreational runners with different fitness levels	60 recreational runners		Fitness level: Better vs. lower	AVR ↑ (p <0.01)	

	Study	Study population and study design	Sample size and response rate	Age range	Physical factors	Retinal arteriolar caliber	Retinal venular caliber
5 2011	Anuradha S MESA	Multi-ethnic Americans; Population-based, cross-sectional study	5887 out of 6814, 86.4%	45-84 yrs	<u>Intentional Physical activity:</u> Lowest quartile (<53 MET-min/wk) vs. highest quartile (≥1838 MET-min/wk) <u>TV viewing time:</u> Highest (>3 hrs/d) vs. lowest quartile (0-1 hrs/d)	n.s. n.s.	220.1 vs. 217.8 µm P<0.05 219.9 vs. 218.2 µm P<0.05
6 2010	Tikellis G ARIC	Caucasian Americans African Americans Population-based, cross-sectional study	12363 out of 15972 77.4%	45-64 yrs	<u>Work Index (1-5):</u> Intermediate working level (2.1-3.0) vs. low working level (1.0-2.0)	n.s.	1.00 µm↓ (-1.74, -0.26)

Abbreviations SCES: Sydney Children Eye Study; MESA: Multi-Ethnic Study of Atherosclerosis, ARIC, the Atherosclerosis Risk in Community; MET-min/wk, metabolic-equivalent/week.

1.3.1.5 Associations with Blood Pressure and Hypertension

The association between peripheral blood pressure and retinal microcirculation in vivo has been repeatedly reported in several large population-based and multi-ethnicity studies. The impacts of systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MABP) on retinal arteriolar caliber are strong and consistent which are both seen in adults[7-9, 28, 37, 38, 59, 60] and children,[27, 61, 62] while the impact on retinal venular calibers are less strong and consistent.

The inverse relationship between elevated blood pressure and retinal arteriolar narrowing has been initially reported in the ARIC study by qualitative assessments, and later reported in BDES, MESA, Funagata study, WESDR, SiMES, SCES and SCROM by quantitative assessments with the development of new retinal image grading methods. In adults, each 10 mm Hg increase in SBP and DBP resulted in a 1.81 μm and a 2.68 μm narrowing in retinal arteriolar caliber in adults by Gepstein et al.[37] and by Wong et al.[9], respectively. Similarly, each 10 mm Hg increase in MABP was associated with a 2.2-4.4 μm narrowing in retinal arteriolar caliber in MESA, WESDR, the Funagata Study and BDES.[7, 38, 59, 60] By using a different unit increase in MABP, Liew et al. (by each standard deviation [SD])[28] and Sun et al. (each interquartile range [IQR])[8] reported a 4.01 and a 6.4 μm narrowing in retinal arteriolar caliber, respectively. In children, Gopinath et al. found a similar magnitude around 5 μm narrowing in retinal arteriolar caliber in Australian adolescents by comparing the highest quartile in SBP/DBP/MABP with the lowest quartile.[61] Each 10 mm Hg increase in SBP and DBP was correlated with a 1.43-2.08 μm and a 2.53

μm narrowing in retinal arteriolar caliber in children recruited from Singapore, Australia and Germany (**Table 7**).[27, 62]

However, the relationship between blood pressure and retinal venular caliber was supported by all the mentioned studies. Only MESA, SCES and WESDR studies reported a 1.8-2.1 μm decrease in retinal venular caliber with each 10 mm Hg increase in MABP[7, 59, 61] while the direction was opposite with a 2.6 μm increase in retinal venular caliber in ARIC by Liew et al.[28] The inconsistency of these results may be due to different adjustments applied in different studies, especially recent studies suggest to add retinal fellow vessels since there might be independent predictive effect of arteriolar versus venular caliber for systemic factors such BP and hypertension.[17, 63] Furthermore, Gopinath B studied 1739 Sydney children in primary school and found that girls with a positive parental history of hypertension had a significantly narrower retinal arteriolar caliber than girls without parental history of hypertension (161.6 vs, 165.9 μm; p=0.0004) (**Table 7**).[64]

The strength of the cross-sectional association between narrower retinal arteriolar caliber and elevated blood pressure has also been shown to vary with age. After adjusting for potential confounders, an interaction between age and blood pressure was found in Rotterdam Study, MESA and BDES on retinal arteriolar caliber.[29, 65, 66] This might reflect the age-dependent progression in rigidity and sclerosis of arterioles that restricts their ability to adequately react to blood pressure changes in the elderly. [29, 65, 66]

With the development of advancing techniques in retinal imaging, more retinal vascular parameters are able to be quantitatively assessed by reliable semi-automatic

computer software. Therefore, the relationship between blood pressure and complementary geometry of retinal vascular network is also explored by different studies. Table 7 shows associations between blood pressure measurements and different types of retinal vascular parameters both in adults, children and adolescents.[35, 67-73] Cheung et al. reported significant associations between elevated blood pressure measurements (SBP/DBP/MABP) and smaller retinal arteriolar tortuosity and larger retinal venular tortuosity in Singapore Malay adults.[72, 73] In this SiMES population, each 10 mm Hg increase in MABP was associated with a $6.4x10^{-6}$ and a $8.4x10^{-6}$ decrease in retinal arteriolar and venular tortuosity, respectively.[72] Similarly, Taarnhoj et al.[67] and Owen et al.[70] also found strong correlation between blood pressure and arteriolar tortuosity both in twins and patients with Type 1 and Type 2 diabetes. However, this association seems to be consistent in the children study. In the Child Heart and Health Study in England (CHASE), each standard deviation increase in multi-ethnic children between 10-11 years was associated with a 2.3% (0.1%, 4.6%) increase in retinal arteriolar tortuosity (**Table 8**).[69] Even though all these studies used objective assessment with good agreement and repeatability, the reason for such inconsistent associations might be multifactorial, such as age and ethnicity difference, variation in retinal vessel grading, bias in choosing statistical models, etc.

Fractal analysis is a method to quantify the geometric branching complexity and density of the retinal vessels. A fractal is a type of geometric pattern that permits the characterization of objects that branch repeatedly, thus, fractal dimension and branching angle are quite highly correlated yet the former represents a more complete

branching pattern for the retinal circulation. Based on this, elevated blood pressure measurements were consistently associated with smaller fractal dimension, followed by smaller retinal arteriolar branching angle and branching asymmetry ratio. Branching asymmetry ratio is defined as the square of the two branching vessel widths ratio, so larger values of such parameter indicates that the widths of the two daughter branching vessels are more symmetric. For instance, each SD increase in SBP was associated with a 0.29 decrease ($p<0.0001$) in fractal dimension in Australian adults aged 49-97 years[35] while each 10 mm Hg increase in MABP was associated with a 0.74 decrease ($p=0.021$) in fractal dimension in Singapore children aged 7-9 years.[71] Similar magnitude of such assocation was also reported in Singapore Malay adult population. Furthermore, Sasongko et al. investigated 1159 Sydney pediatric patients with type 1 diabetes and found a strong relationship between elevated SBP and smaller retinal arteriolar length/diameter ratio (LDR) ($p=0.002$) (**Table 8**). [68]

From all this large population-based, epidemiological studies on different ethnicities and ages, we can conclude that blood pressure is a vital risk factor for changes in retinal vascular caliber and other parameters, mainly on retinal arterioles. The vascular network is believed to be organized to minimize shear stresses and work across the system, and these parameters may provide an indication of how closely a given network conforms to the geometrical ideal. Geometric network measures provide a complete indication of the overall health of the vascular system. Therefore, narrower retinal arteriolar caliber, smaller retinal arteriolar tortuosity, smaller retinal arteriolar branching angle and smaller retinal fractal dimension form a series of

abnormal polymorphism of retinal circulation network, provided with the elevation of peripheral blood pressure. This might help to provide substantial evidence in the pathophysiological mechanism of hypertensive retinopathy and arteriolar remodeling. On the contrary, the relationship between blood pressure and retinal venules is less strong and consistent than that between blood pressure and retinal arterioles. It might be explained that retinal venules are not as sensitive as retinal arterioles according to the regulation of blood pressure.

Table 7. Associations between Blood Pressure, Hypertension and Retinal Vascular Caliber

	Study	Study population and study design	Sample size and response rate	Age range	Blood pressure	Retinal arteriolar caliber	Retinal venular caliber
1 2012	Hanssen H JuvenTUM 3 2008	Germany school children at 5th grade School-based cross-sectional study	578 out of 792 73.0%	Mean age 11.1 yrs	DBP: each 10 mmHg↑	2.53 ↓ (p=0.002)	n.s.
2 2011	Gopinath B SCES	Sydney primary school pupils from year 1 to year 6 Multi-ethnicity Cross-sectional	1739 out of 2238 77.7%	mean age: 6.7 yrs	Parental HTN: with vs. without among girls only	161.6 vs. 165.9 µm (p=0.0004)	n.s.
3 2010	Gopinath B SCES	Australian adolescents Population-based, cross-sectional study	2353 out of 3144 75.3%	Mean age 12.7 yrs	SBP, DBP, MABP highest vs. lowest Quartile	~ 5 µm ↓ (p trend<0.0001)	~ 2.0 µm ↓ (p=0.002)
4 2009	Jeganathan V MESA	Population-based, cross-sectional study	3749	24+ yrs	MABP: each 10 mmHg↑ HTN: uncontrolled vs. controlled vs. non	3.1 µm ↓ (p<0.0001) 140.0 vs. 142.1 vs. 146.0 µm (p<0.0001)	1.8 µm ↓ (p<0.0001) n.s.
5 2008	Sun C SiMES	Singapore Malays Population-based, cross-sectional study	3019 out of 3280 92.0%	40-80 yrs	MABP: each SD ↑	4.01 µm ↓ (p<0.001)	n.s.

	Study	Study population and study design	Sample size and response rate	Age range	Blood pressure	Retinal arteriolar caliber	Retinal venular caliber
6 2008	Liew G ARIC	White and African Americans, Population-based, cross-sectional study	8794 out of 12887, 68.2%	48-73 yrs	MABP: each IQR ↑	6.4 μm ↓ (p<0.05)	2.6 μm ↑ (p<0.05)
7 2007	Mitchell P SCES, SCORM	Australians children Singapore children School-based, cross-sectional study	1572 Australian children 380 Singapore children	SCES: 6-8 yrs SCORM: 7-9 yrs	SBP: each 10mmHg ↑	SCES: 2.08 μm ↓ (p<0.0001) SCORM: 1.43 μm↓ (p=0.016)	n.s.
8 2006	Klein R WESDR	370 with diabetes diagnosed with 30+ years Population-based, cross-sectional study	1370 with type 2 DM	30+ yrs	MABP: each 10mmHg ↑	2.2 μm ↓ (P<0.05)	2.1 μm ↓ (p<0.05)
9 2006	Wong TY MESA	Americans Population-based cross-sectional study	5979	45-84 yrs	SBP: each SD ↑	2.68 μm ↓ (p<0.001)	n.s.
10 2005	Kawasaki R The Funagata Study	Japanese participants Population-based, cross-sectional study	1961 out of 3676 53.3%	≥35 yrs	MABP: each 10mmHg ↑	2.8 μm ↓ (p<0.05)	n.s.
11 2003	Wong TY BDES	American patients Population-based, cross-sectional study	4926	43-84 yrs	MABP: each 10mmHg↑	4.4 μm ↓ (95% CI: 3.8, 5.0)	n.s.

Abbreviations: SCES: Sydney Children Eye Study; MESA: Multi-Ethnic Study of Atherosclerosis; SiMES: Singapore Malay Eye Study; ARIC, the Atherosclerosis Risk in Community; SCORM: the Singapore Cohort Study of the Risk Factors of Myopia; WESDR, Wisconsin Epidemiological Study of Diabetic Retinopathy; BMES, Blue Mountains Eye Study; BEDS: Blue Mountains Eye Study.

Table 8. Associations between Blood Pressure and Retinal Vascular Tortuosity, Branching Angles and Fractal Dimension

	Study	Study population and study design	Sample size and response rate	Age range	Blood pressure	Retinal arteriolar characteristics	Retinal venular characteristics
1 2011	Owen C CHASE	Cross-sectional study	968 UK children	10-11 years	DBP: each SD ↑	**Arteriolar tortuosity:** 2.3% ↑ (0.1%, 4.6%)	n.s.
2 2011	Cheung CY SiMES	Population-based, cross-sectional study	3280 participants	40-80 years	MABP: each 10mmHg↑	**Arteriolar tortuosity:** $6.4x10^{-6}$↓ **Fractal dimension** 0.10↓	**Venular tortuosity:** $8.4x10^{-6}$↑
3 2011	Cheung CY SiMES	Singapore Malays Population-based, cross-sectional study	1913 out of 3280 58.3%	40-80 years	SBP/DBP/MABP: highest vs. lowest quintile	**Arteriolar tortuosity:** ↓ ($p<0.001$) **Arteriolar branching angle** ↓ ($p<0.05$) **Arteriolar branching asymmetry ratio** ↑ ($p<0.05$) **Fractal dimension** ↓ ($p=0.001$)	**Venular tortuosity:** ↑($p=0.021$) n.s. n.s.
4 2011	Kurniawan ED SCORM	Singapore school children Cross-sectional	1979 out of 2913 67.9%	10-14 years	MABP: each 10mmHg ↑ DBP: each 10mmHg ↑	**Fractal dimension:** 0.74 ↓ ($p=0.021$) **Fractal dimension:** 0.13↓ ($p=0.022$)	

	Study	Study population and study design	Sample size and response rate	Age range	Blood pressure	Retinal arteriolar characteristics	Retinal venular characteristics
5 2010	Sasongko MB Sydney Pediatric Diabetes Study	Sydney children and adolescents with type 1 diabetes Cross-sectional study	944 out of 1159 81.4%	12-20 yrs	SBP: ↑	**Arteriolar LDR** ↓ (p=0.002)	n.s.
6 2008	Liew G BMES	Population-based, cross-sectional study	3654 patients	49-97 yrs	SBP: each SD ↑	**Fractal dimension:** 0.29 ↓ (p<0.0001)	
7 2008	Taarnhoj NCBB	Cross-sectional	57 Monozygotic twin 52 dizygotic twin	20-46 yrs	BP: ↑	**Arterial tortuosity** ↓	n.s.
8 2007	Owen C	Case control study	17 Type 1 DM 36 Type 2 DM 60 controls	20-94 yrs	MABP: each 20 mmHg↑	**Overall tortuosity** Type 1 DM vs. controls 8.2%↓ (-13.3%, -3.0%) Type 2 DM vs. controls 6.4%↓ (-10.9%, -1.9%)	

Abbreviation: CHASE: The Child Heart and Health Study in England; SiMES: Singapore Malay Eye Study; SCORM: the Singapore Cohort Study of the Risk Factors of Myopia; BMES, Blue Mountains Eye Study.

1.3.1.6 Associations with Anthropometric Parameters and Obesity

Anthropometric parameters include weight, height, body mass index (BMI), subcutaneous skinfold thickness, body surface area, waist circumference, upper mid-arm circumference, waist-to-hip ratio and so on. All these indexes have been used to assess the body fatness composition so as to evaluate whether a person is healthy or not. According to the WHO guidelines, BMI is the gold standard index used in identifying body fat status among general population, which is underweight (BMI<18.5 kg/m^2), normal weight (BMI=18.5-24.9 kg/m^2), overweight (BMI=25.0-29.9 kg/m^2) and obesity (BMI≥30.0 kg/m^2).[74]

Since it is well-known that diabetes can cause diabetic retinopathy, researchers have been investigating the ocular effects[75] due to obesity or even early abnormal anthropometric measurements. In three population-based or school-based cross-sectional studies in Germany,[27] Australia[6, 76] and Singapore,[62] children with higher BMI tended to have abnormal retinal vascular signs. In the JuvenTUM 3 study of 578 children with mean age 11.1 years, obese children had a smaller AVR than those with normal weight (0.85 vs. 0.89, $p<0.05$).[27] Similarly, in Australian adolescents with mean age 12.7 years, obese subjects had a 2.8 μm ($p=0.01$) narrowing in retinal arteriolar caliber and a 4.5 μm ($p=0.01$) widening in retinal venular caliber compared with subjects with normal weight, respectively.[6] Furthermore, in the same study of different age groups, children above 85th percentile of the BMI growth chart had a 1.7 μm ($p=0.003$) narrower retinal arteriolar caliber and a 2.7 μm ($p=0.001$) wider retinal venular caliber, respectively.[76] In Asian children from Singapore aged 7-9 years, each

standard deviation increase in BMI was associated with a 2.55 μm ($p<0.001$) widening in retinal venular caliber (**Table 9**).[62]

As for adults, the direction of this association between BMI and retinal venular caliber remains consistent yet the magnitude of retinal venular caliber changes seems slightly smaller. In four population-based adult studies, increase in BMI was associated with increase in retinal venular caliber.[7, 9, 28, 77] Since the linear regressions were used differently across these studies, we couldn't compare the magnitudes of retinal venular caliber changes directly. However, the relationship between BMI and retinal arteriolar caliber was only suggested in ARIC by Liew et al.[28] but not in the Funagata Study, WESDR and MESA (**Table 9**).[7, 9, 77] This might imply that obesity or body fatness have a more sensitive impact on retinal venular caliber rather than retinal arteriolar caliber, which are supported by the associations found between other fatness indices and retinal venular caliber. For example, each standard deviation increase in waist circumference was associated with a 0.99 μm increase and a 3.73 μm increase in retinal venular caliber in Australia children age 6 years[76] and Japanese adults aged 35 years and above,[77] respectively. Moreover, each standard deviation increase in body surface area was associated with a 1.97 μm increase in retinal venular caliber in 6-year-old Australian children.[76]

The associations between BMI and retinal geometric parameters are barely investigated. Up till now there have only been two studies suggesting the relationship between BMI and retinal arteriolar tortuosity.[67, 72] Cheung[72] and Taarnhøj[67] both reported an association between larger BMI and lower retinal arteriolar tortuosity among Singapore Malays and Danish Caucasian twins (**Table 10**).

Table 9. Associations between Anthropometric Parameters, Obesity and Retinal Vascular Caliber

	Study	Study population and study design	Sample size and response rate	Age range	Anthropometric parameters	Retinal arteriolar caliber	Retinal venular caliber
1 2012	Hanssen H JuvenTUM 3	Germany school children at 5th grade School-based cross-sectional study	578 out of 792 73.0%	Mean age 11.1 years	Obese vs. normal wt Overweight vs. normal wt	AVR: 0.85 vs. 0.89 (p<0.001) AVR: 0.87 vs. 0.89 (p =0.03)	
2 2011	Gopinath B SCES	Australian adolescents Population-based, cross-sectional study	2353 out of 3144 75.3%	Mean age 12.7 years	BMI: 4th vs. 1st quartile Obese vs. normal wt	2.8 µm ↓ (p<0.0001) 2.8 µm ↓ (p=0.01)	4.2 µm ↑ (p=0.001) 4.5 µm ↑ P=0.01
3 2008	Liew G ARIC	White and African Americans Population-based, cross-sectional study	8794 out of 12887 68.2%	48-73 years	BMI: each IQR ↑	0.7 µm ↓ (-1.1, -0.3) (p=0.001)	0.6 µm ↑ (0.2, 1.0) (p<0.0001)
4 2007	Taylor SCES	Population-based, cross-sectional study	1608 out of 1740 92.4%	6 years	BMI: each SD ↑ BMI: above vs. below threshold Waist circumference: each SD ↑ Body surface area: each SD ↑	0.76 µm↓ (-1.43, -0.08) 162.0 vs. 163.7 µm (p=0.003) n.s. n.s.	1.13 µm ↑ (0.11, 2.15) 231.7 vs. 229.0 (p=0.001) 0.99 µm ↑ (0.15, 1.84) 1.97 µm ↑ (0.86, 3.09)

	Study	Study population and study design	Sample size and response rate	Age range	Anthropometric parameters	Retinal arteriolar caliber	Retinal venular caliber
5 2007	Kawasaki R The Funagata Study	Japanese patients Population-based, cross-sectional study	1961	35+ years	Waist circumference: each SD ↑	n.s.	3.73 μm ↑ (0.72, 6.76)
6 2006	Cheung N SCORM	Singapore Chinese School-based, cross-sectional study	768	7-9 years	BMI: each SD ↑	n.s.	2.55 μm ↑ (p<0.001)
7 2006	Klein R WESDR	370 with Type 2 diabetes Population-based, cross-sectional study	1370	30+ years	BMI: each 1kg/m^2 ↑	n.s.	0.3 μm ↑ (p=0.007)
8 2006	Wong TY MESA	Americans Population-based cross-sectional study	5979	45-84 years	BMI: each SD ↑	n.s.	2.21 μm ↑ (p<0.001)

Abbreviation: SCES: Sydney Children Eye Study; ARIC: the Atherosclerosis Risk In Community; SCORM: the Singapore Cohort Study of the Risk Factors of Myopia; WESDR: Wisconsin Epidemiological Study of Diabetic Retinopathy; MESA: Multi-Ethnic Study of Atherosclerosis.

Table 10. Associations between Anthropometric Parameters and Retinal Vascular Tortuosity, Branching Angles and Fractal Dimension

	Study	Study population and study design	Sample size and response rate	Age range	Anthropometric parameters	Retinal arteriolar characteristics	Retinal venular characteristics
1 2011	Cheung CYL SiMES	Singapore Malay Population-based, cross-sectional study	2915 out of 3280 88.8%	40-80 years	BMI: ↑	Retinal arteriolar tortuosity↓ ($p<0.05$)	n.s.
2 2008	Taarnhoj NCBB The Geminakar Study	Cross-sectional	57 Monozygotic twin 52 dizygotic twin	20-46 years	BMI: 0.06 kg/m^2 ↓	Retinal arterial tortuosity: One-step ↑ ($p<0.05$)	n.s.

Abbreviation: SiMES: Singapore Malay Eye Study

1.3.1.7 Associations with Markers of Inflammation and Dyslipidaemia

Inflammation, endothelial dysfunction and dyslipidaemia are now considered as major underlying mechanisms of both large and small vascular diseases.[78, 79] A body of evidence has shown that systemic inflammatory markers (e.g. high sensitive C-reactive protein [hs-CRP], white blood cell counts, interleukin 6 [IL-6], serum homocysteine, serum amyloid A [SAA], serum albumin, etc.) are associated with retinal venular caliber and/or retinal arteriolar caliber.[9, 27, 28, 80-83] There's a consistent finding on the association between higher hs-CRP and retinal venular widening in both children and adult studies.[9, 27, 81, 83] Compared with hs-CRP in the lowest quartile, adults classified into the highest quartile of hs-CRP had a range of 5.2 to 11.3 μm in retinal venular widening (**Table 11**).[9, 83] Furthermore, other bio-markers implicating higher inflammatory status such as lower level of serum albumin, lower level of homocysteine,[9, 80] higher level of white cell count,[28] higher level of IL-6[9] and higher level of serum amyloid A,[83] were found to be associated with retinal arteriolar narrowing and/or retinal venular widening. For example, each SD increase in homocysteine was associated with a 1.53 μm ($p=0.03$) and a 3.78 μm ($p<0.05$) widening in retinal venular caliber in BMES[82] and Hoorn Study,[80] respectively. Compared with the lowest quartile, highest quartile of white cell count and serum amyloid A had a 0.9 μm (p trend <0.0001) and a 10.2 μm (p trend=0.003) increase in retinal venular caliber, respectively.[28, 83]

As for endothelial dysfunction, systemic markers such as soluble intercellular adhesion molecule-1 (sICAM-1), plasma fibrinogen and von willebrand factor (vwf) are the most well studied in their relationships with retinal vascular caliber. Higher

level of sICAM-1 and Fibrinogen were suggested to be associated with retinal venular widening,[9, 28] and higher level of vwf and fibrinogen were suggested to be associated with retinal arteriolar widening in ARIC and MESA studies (**Table 11**).[9, 28]

Moreover, biomarkers of dyslipidaemia like high-density lipoprotein (HDL) cholesterol (CHOL), low-density lipoprotein (LDL) cholesterol and triglycerides (TG) were repeatedly found by quite a few population-based studies to be associated with retinal arteriolar narrowing and/or retinal venular widening.[8, 9, 28, 37] Each SD increase HDL increase was associated with a 1.13 μm and 1.71 μm narrowing in retinal venular caliber in SiMES[8] and MESA[9] participants. On the contrary, each SD or IQR increase in LDL was associated with a 0.93 μm or a 0.9 μm increase in retinal venular caliber in American adults, respectively.[9, 28] Consistent with LDL, higher level of triglyceride was consistently associated with retinal venular widening.[9] These data might implicate that these relationships between dyslipidaemia and retinal vascular caliber changes could involve inflammation and endothelial dysfunction.

Only a few studies have been performed on relationships between such markers and retinal geometric parameters. Stettler et al. investigated 711 patients from Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) and reported positive relationship of higher level of inflammation markers (SAA and CRP) with larger retinal arteriolar Length-to-Diameter Ratio (LDR) both in diabetic and non-diabetic patients, and with larger retinal arteriolar tortuosity only in non-diabetic patients.[84] Higher level of markers of dyslipidaemia (TG, CHOL, LDL) were found to be associated with more tortuous retinal arterioles in UK children,[69] and lower level of HDL was associated with more tortuous retinal venules in Singapore Malay adults (**Table 12**).[11]

Table 11. Association between Markers of Inflammation, Endothelial Function and Dyslipidaemia and Retinal Vascular Caliber

	Study	Study population and study design	Sample size and response rate	Age range	Markers	Retinal arteriolar caliber	Retinal venular caliber
1 2012	Gepstein B Clinical study	Annual check-up patients Hospital-based, cross-sectional study	285 patients	33-87 years	Dyslipidaemia: Yes vs. No	0.189 µm ↑ (p=0.004)	n.s.
2 2012	Hanssen H JuvenTUM 3	Germany school children at 5th grade School-based cross-sectional study	578 out of 792 73.0% 122 overweight or obese	Mean age 11.1 years	hs-CRP: each 1 unit ↑	n.s.	11.01 µm ↑ (p=0.002)
3 2009	Cheung CYL Singapore Prospective Study Program	Chinese, Malays, Indians Population-based cross-sectional study	3583 out of 7744 46.3%	24-95 years	hs-CRP: highest vs. lowest quartile	n.s.	3.94 µm ↑ (P trend<0.001).
4 2009	Gopinath B BMES	Population-based cross-sectional study	2334 participants	49+ years	Homocysteine: each SD ↑	1.53 µm ↑ (p=0.03) Only in men	n.s.
5 2008	Sun C SiMES	Singapore Malays Population-based, cross-sectional study	3019 out of 3280 92.0%	40-80 years	HDL level: each SD ↑	n.s.	1.71 µm ↓ (p<0.001)

	Study	Study population and study design	Sample size and response rate	Age range	Markers	Retinal arteriolar caliber	Retinal venular caliber
6 2008	Van Hecke Hoorn Study 2000-2001	Caucasians Population based, cross-sectional study	2484	50-74 years	Homocysteine: each SD ↑	3.78 µm ↓ (p<0.05)	n.s.
7 2008	Liew G ARIC	White and African Americans Population-based, cross-sectional study	8794 out of 12887 68.2%	48-73 years	Serum albumin: each IQR ↑	1.5 µm ↑ (p<0.0001)	n.s.
					White cell: each IQR ↑	n.s.	0.9 µm ↑ (p<0.0001)
					Von willebrand factor: each IQR ↑	0.9 µm ↑ (p=0.001)	0.8 µm ↓ (p=0.0002)
					Fibrinogen: each IQR ↑	n.s.	0.7 µm ↑ (p=0.0002)
					HDL: each IQR ↑	0.5 µm ↓ (p=0.02)	n.s.
					LDL: each IQR ↑	n.s.	0.9 µm ↑ (p<0.0001)
8 2006	Klein R BDES	Population-based, cross-sectional study	396	50-86 years	hs-CRP: 1st vs.4th quartile	n.s.	11.3 µm↑ (p trend<0.001)
					Serum amyloid A: 1st vs. 4th quartile	8.2 µm↑ (p trend<0.001)	10.2 µm↑ (p trend=0.003)

	Study	Study population and study design	Sample size and response rate	Age range	Markers	Retinal arteriolar caliber	Retinal venular caliber
9 2006	Wong TY MESA	Americans Population-based cross-sectional study	5979	45-84 years	Homocysteine: 1st vs. 4th quartile	1.3 µm ↓ (p trend=0.03)	n.s.
					Hs-CRP: 1st vs. 4th quartile	n.s.	5.2 µm ↑ (p trend<0.001)
					IL-6: 1st vs. 4th quartile	n.s.	6.1 µm ↑ (p trend<0.001)
					Fibrinogen: 1st vs. 4th quartile	1.2 µm ↑ (p trend=0.02)	6.1 µm ↑ (p trend<0.001)
					sICAM-1: 1st vs. 4th quartile	n.s.	4.1 µm ↑ (p trend=0.01)
					Triglycerides: 1st vs. 4th quartile	n.s.	5.7 µm ↑ (p trend<0.01)
					HDL: each SD ↑	n.s.	1.13 µm ↓ (p=0.001)
					LDL: each SD ↑	n.s.	0.93 µm ↑ (p=0.02)

Abbreviation: BMES: Blue Mountains Eye Study; SiMES: Singapore Malay Eye Study; ARIC: Atherosclerosis Risk in Community; BDES: Beaver Dam Eye Study; MESA: Multi-Ethnic Study of Atherosclerosis.

Table 12. Association between Markers of Inflammation, Endothelial Dysfunction and Dyslipidaemia and Retinal Vascular Tortuosity, Branching Angle and Fractal Dimension

	Study	Study population and study design	Sample size and response rate	Age range	Markers	Retinal arteriolar characteristics	Retinal arteriolar characteristics
1 2011	Owen C CHASE	Cross-sectional study	968 UK children	10-11 years	TG: each SD ↑	Tortuosity: 3.7% ↑ (1.2%, 6.4%)	n.s.
					Total cholesterol: each SD ↑	Tortuosity: 3.3% ↑ (0.9%, 5.8%)	n.s.
					LDL: each SD ↑	Tortuosity: 3.1% ↑ (0.6%, 5.6%)	n.s.
2 2011	Cheung CYL SiMES	Singapore Malay Population-based, cross-sectional study	2915 out of 3280 88.8%	40-80 years	HDL: ↓	n.s.	Tortuosity: ↑ ($p<0.05$)
4 2009	Stettler C ASCOT	Cross-sectional study	711 patinets (159 w/t DM; 552 w DM)	>55 years	SAA: 1st vs. 3rd tertile	L:D ratio: ↑ DM & non-DM ($p<0.05$) Tortuosity: ↑ in non-DM ($p=0.012$)	n.s. n.s.
					CRP: 1st vs. 3rd tertile	Tortuosity: ↑ in non-DM ($p=0.039$)	n.s.

Abbreviation: CHASE: the Child Heart and Health Study in England; SPDS: Sydney Pediatric Diabetes Study; ASCOT: An Anglo-Scandinavian Cardiac Outcomes Trial.

1.3.1.8 Association with Ocular Anatomic Structure

There is increasing evidence showing associations between retinal circulation and eye diseases.[85] In order to further inveistigate the pathological cause of eye diseases, it is necessary to study the relationship of structural markers of retinal circulation with early hallmark of optical disorders, such as thinner retinal nerve fiber layer (RNFL) thickness and higher intraocular pressure (IOP).

The most studied ocular anatomic structures are RNFL thickness, optic disk cup-to-disc ratio, optic disc rim area and macular total volume.[73, 85-95] In children, each SD increase in RNFL thickness was reported to be associated with an increase of 0.22-0.62 μm in retinal arteriolar caliber and an increase of 0.49-0.99 μm in retinal venular caliber, respectively independent of ethnicity and refractive status.[87-89, 91, 94] In adults, the magnitudes seemed to be much larger with each SD decrease in global RNFL thickness (a 5.81 μm decrease in retinal arteriolar caliber and a 8.37 μm decrease in retinal venular caliber, respectively),[85] which might be due to nature of the optical growth. The relationship between RNFL and retinal venular caliber remained significant in patients with glaucoma (**Table 13**).[85]

Similarly, other optical structures like larger cup-to-disc ratio, larger optic rim area, macular volume and rim-to-disc area (RDA) were consistently associated with larger retinal vascular caliber and larger retinal vascular tortuosity both in children and adults.[85-87, 89-94] Based on new understanding that central thickness (CCT) being a surrogate marker for glaucoma susceptibility, corneal biomechanical properties such as corneal hysteresis (CH) and corneal resistance factor (CRF) were also studied and

found to be positively associated with retinal arteriolar caliber.[91] Furthermore, as a direct hallmark of glaucoma, each 1 mm Hg increase in IOP was associated with a decrease of 0.2 μm and 0.5 μm in retinal arteriolar and venular caliber respectively,[7] which strongly supported the hypothesis of impact of a series of glaucomatous biomechanical changes on retinal circulation. For instance, such defects on retinal vessels (narrowing retinal arterioles) could lead to disproportionate release of endogenous vasodilators (e.g., nitric oxide and prostaglandin I2) and/or vasoconstrictors (e.g., endothelin), which could further result in optic nerve ischemia, and ultimately into glaucomatous optic neuropathy (**Table 13**).[85]

In contrast from the association between glaucomatous surrogates and retinal vascular parameters, the findings of associations between myopic surrogates and retinal circulation were inconsistent. In Asian adults, elongated axial length or worse spherical equivalent (SE) were suggested to be associated with retinal arteriolar and venular narrowing, less retinal arteriolar and venular tortuosity and higher branching coefficient even after corrected for ocular magnification.[92] In Asian children and Caucasian adults, refractive error was not associated with either retinal vascular caliber or retinal vessel fractal dimension.[73, 88, 95] Lim et al. speculated that straighter (less tortuous) retinal vessels and correspondingly more acute bifurcations along with longer axial length might be consistent with mechanical stretch on the vessels in the posterior pole as the eye elongates posteriorly due to myopia.[92] These changes may in turn have implications for decreased ocular pulse or lower ocular blood flow in myopic eyes, which have been examined by Doppler ultrasound (**Table 14**).[96-98]

Table 13. Associations between Ocular Anatomic Structures and Retinal Vascular Caliber

	Study	Study population and study design	Sample size and response rate	Age range	Ocular structure	Retinal arteriolar caliber	Retinal venular caliber
1 2010	Lim L SiMES	Singapore Malays School-based, cross-sectional study	2882	40-80 years	Axial length: each 1 mm↑	3.20 μm ↓ (p<0.001)	5.21 μm ↓ (p<0.001)
					SE: each 1 D ↑	0.42 μm ↑ (p<0.001)	0.69 μm ↑ (p<0.001)
3 2010	Chang MW Clinical study	60 patients with normal-tension glaucoma (NTG) 45 age-sex-matched healthy controls	60 patients with untreated NTG	30-70 years	Retinal Nerve Fiber Layer (RNFL): each SD ↑	0.38 ↑ μm p=0.01	n.s.
4 2009	Lim L SCORM	Singapore children Observational cross-sectional study	104	Mean age 11.51 years	Horizontal Integrated Rim width: each SD ↑	9.96 μm ↑ (p=0.04)	21.16 μm ↑ (p=0.002)
					RNFL: each SD ↑	0.26 μm ↑ (p=0.04)	0.49 μm ↑ (p=0.009)
					Larger cup-to-disc ratio: each SD ↑	n.s.	26.69 μm ↑ (p=0.003)
					Larger total macular volume: each SD ↑	n.s.	12.54 μm ↑ (p=0.02)

	Study	Study population and study design	Sample size and response rate	Age range	Ocular structure	Retinal arteriolar caliber	Retinal venular caliber
5 2009	Lim L SCORM	Singapore children Observational cross-sectional study	257 out of 1979	Mean age 13.96 years	Corneal hysteresis (CH): each SD ↑	1.40 µm↑ (p=0.03)	n.s.
					Corneal resistance factor (CRF): each SD ↑	1.68 µm ↑ (p=0.03)	n.s.
6 2009	Samarawickrama C SCES	Population-based, cross-sectional study	2038 children	Mean age 12 years	RNFL: each unit ↑	0.22 µm ↑ P<0.0001	0.49 µm ↑ (p=<0.0001)
					Macular volume each unit ↑	5.31 µm ↑ (p<0.0001)	10.95 µm ↑ (p<0.0001)
					Optic disc area: each unit ↑	2.02 µm ↑ (p=0.01)	5.02 µm ↑ (p<0.0001)
					Optic disc rim area: each unit ↑	1.81 µm ↑ (p=0.01)	2.90 µm ↑ (p=0.005)
7 2009	Zheng YF SiMES	Population-based, cross-sectional study	2706 patients	40-80 years	Global RNFL thickness: each SD ↓	5.81µm↓ (p<0.001)	8.37µm↓ (p<0.001)

	Study	Study population and study design	Sample size and response rate	Age range	Ocular structure	Retinal arteriolar caliber	Retinal venular caliber
7 2009	Zheng YF SiMES	Population-based, cross-sectional study	2706 patients	40-80 years	Temporal region: each SD ↓ in glaucoma Temporal-to-inferior region: each SD ↓ in glaucoma	n.s. n.s.	8.54μm↓ (p=0.022) 38.32 μm↓ (p=0.006)
8 2008	Cheung N SCES	Sydney Childhood Eye Study Populationbased cross-sectional study	1204 healthy children	Mean age 6 years	Optic disc area: each SD ↓ Optic cup area: each SD ↓ Macular total volume: each SD ↓ RNFL: each SD ↓	0.14 μm ↓ (p=0.05) 0.15 μm ↓ (p=0.05) 0.36 μm ↓ (p<0.01) 0.62 μm ↓ (p<0.01)	0.31 μm ↓ (p<0.01) 0.43 μm ↓ (p<0.01) 0.62 μm ↓ (p<0.01) 0.99 μm ↓ (p<0.01)
9 2007	Cheung N SCORM	Singapore Chinese children School-based cross-sectional study	746 out of 1979	7-9 years	Vertical disc diameter: each unit ↓	0.009 pixel ↓ (p=0.02)	0.014 pixel ↓ (p=0.01)

	Study	Study population and study design	Sample size and response rate	Age range	Ocular structure	Retinal arteriolar caliber	Retinal venular caliber
10 2007	Lee K Beaver Dam Eye Study	Population-based, cross-sectional study	3887 eyes	43-86 years	<u>Horizontal disc</u>: 1st vs. 3rd tertile	164.7 vs. 165.9 µm (p=0.04)	241.2 vs. 243.1µm (p=0.02)
					<u>Vertical disc:</u> 1st vs. 3rd tertile	160.3 vs. 165.8 µm (p<0.001)	235.3 vs. 243.0µm (p<0.001)
					<u>Horizontal cup</u>: 1st vs. 3rd tertile	162.1 vs. 165.8 µm (p=0.02)	n.s.
11 2006	Klein R WESDR	370 with diabetes diagnosed with 30+ years Population-based, cross-sectional study	1370 with type 2 DM	30+ years	<u>IOP</u>: each 1 mm Hg↑	0.2 µm ↓ (p<0.05)	0.5 µm ↓ (p<0.05)
12 2004	Wong TY BMES	Population-based, cross-sectional study	3654 49+ years		<u>SE:</u>	Not associated with vascular caliber	

2. Abbreviation: SiMES: Singapore Malay Eye Study; SCORM: the Singapore Cohort Study of the Risk Factors of Myopia; BDES: Beaver Dam Eye Study; WESDR: Wisconsin Epidemiological Study of Diabetic Retinopathy.

Table 14. Associations between Ocular Anatomic Structure and Retinal Vascular Tortuosity, Branching Angle and Fractal Dimension

	Study	Study population and study design	Sample size and response rate	Age range	Risk factor	Retinal arteriolar characteristics	Retinal venular characteristics
1 2010	Koh V SiMES	Population-based, cross-sectional study	3280 participants	40-80 years	RDA: $7.79x10^{-6}$↓ (p=0.006)	Tortuosity: Per SD ↓	
					RDA: $9.43x10^{-6}$↓ (p=0.001)		Tortuosity: Per SD ↓
2 2010	Lim L SiMES	Singapore Malays School-based, cross-sectional study	2882	40-80 years	Axial length: each 1 mm ↑	Tortuosity : 0.15 $x10^4$↓ (p<0.001)	Tortuosity: 0.122 $x10^4$ ↓ (p<0.001)
						Branching coefficient: 0.027 ↑ (p<0.001)	Branching coefficient: 0.008 ↑ (p<0.02)
					SE: each 1 D ↑	Tortuosity : 0.065 $x10^4$ ↑ (p<0.001)	Tortuosity : 0.006 $x10^4$↓ (p<0.001)
4 2010	Li Haitao BMES	Population-based, cross-sectional study	3654 patients	49-97 years	SE:	Not associated with fractal dimension	

3. Abbreviation: SiMES: Singapore Malay Eye Study; BMES: Blue Mountains Eye Study.

1.3.2 Systemic Diseases with Retinal Microvasculature

Growing evidence has shown that variations in retinal vascular parameters are associated with a range of systemic diseases. Due to the adverse quality of life caused by high morbidity of vascular diseases among all populations, the relationships between retinal vascular parameters and diseases have become essentially important for studying the pathophysiological mechanisms of different diseases.

1.3.2.1 Associations with Hyperglycemia, Diabetes and Metabolic Syndromes

Metabolic syndromes including hyperglycemia, impaired fasting glucose (IFG) and diabetes mellitus (DM) have been long suggested to result in vascular complications in eyes, kidney and peripheral vessels. Studies on relationship between microcirculation *in vivo* and metabolic symptoms and syndromes before might help to explain early manifestation of pathogenic features.

Increased concentration of fasting plasma glucose (FPG) has been repeatedly suggested to be associated with retinal arteriolar widening both in diabetic patients and general population.[9, 99, 100] Each 1 mmol/L increase in FPG was associated with a 0.51 μm increase in retinal venular caliber in Multiethnic Asian population,[100] and each SD increase in FPG was associated with a 0.89 μm and a 1.73 μm increase in retinal arteriolar and venular caliber in MESA, respectively.[9] Similar to fasting glucose level, higher HbA1C level was found to be associated with retinal arteriolar widening and more tortuous retinal arterioles in Singapore Indians.[68, 99] In diabetic and IFG patients, retinal arteriolar caliber and/or retinal venular caliber were found to be significantly wider than non-diabetic patients in SINDI, MESA, BMES and a

clinical study conducted on adolescents by Bronson-Castain et al.[9, 99, 101-103] On the contrary, diabetic patients had a narrower retinal arteriolar caliber in multiethnic Asian population.[100] Furthermore, increased DM duration was associated with larger retinal branching angle,[68] larger optimality deviation,[68] higher fractal dimension[104] and decreased overall retinal tortuosity. [70]

As for a general medical term for hyperglycemia, IFG, diabetes and even other risk factors such as hyperlipidemia and obesity, metabolic syndrome was found to be significantly related to retinal arteriolar caliber and retinal venular caliber changes.[105-107] However, the direction of association between retinal arteriolar caliber and metabolic syndrome is different from that between retinal arteriolar caliber and diabetes. For example in 1003 Japanese adults, each SD decrease in retinal arteriolar caliber and each SD increase in retinal venular widening were associated with 1.57 times (95% CI: 1.30, 1.90) and 1.47 times (95% CI: 1.23, 1.76) of getting metabolic syndrome, respectively.[107] In 869 Chinese subjects, the lowest quartile of retinal arteriolar caliber had 1.78 times (95% CI: 1.02, 3.10) in getting metabolic syndrome compared to the highest quartile of retinal arteriolar caliber.[105] In American participants with metabolic syndrome had 1.23 times (95% CI: 1.12, 1.35) risk of generalized arteriolar narrowing and 1.30 times (95% CI: 1.18, 1.48) risk of generalized venular dilation.[106]

The inconsistency of diabetic patients having wider retinal arteriolar caliber while patients with metabolic syndromes having narrower retinal arteriolar caliber was unknown. However, both diabetes and metabolic syndromes are associated with retinal venular caliber, which have demonstrated that administration of intravenous

dextrose can cause dilatation of retinal venule in both conditions.[1] Moreover, reduced vascular reactivity associated with endothelial dysfunction and inflammatory processes may also play a role in the development of wider retinal venules in people with hyperglycemia, IFG, diabetes and metabolic syndromes.[1]

Table 15. Associations between Metabolic Symptoms and Metabolic Syndromes and Retinal Vascular Caliber

	Study	Study population and study design	Sample size and response rate	Age range	Metabolic factors	Retinal arteriolar caliber	Retinal arteriolar caliber
1 2011	Tsai ASH SINDI	Population-based, cross-sectional study	3400 Indians 980 with DM 327 with DR	40-80 years	DM: Yes vs. No	145.23 vs. 142.38 µm (p<0.001)	n.s.
					Glu/HbA1C: ↑	↑	n.s.
2 2011	Saito K	Hospital-based, cross-sectional study	1003 Japanese adults		Metabolic syndrome: OR: 1.57 (1.30, 1.90)	Per SD ↓	n.s.
					Metabolic syndrome: OR: 1.47 (1.23, 1.76)	n.s.	Per SD ↑
3 2010	Yuan YZ High-risk for Diabetes Changfeng Study	Hospital-based, cross-sectional study	286 with metabolic syndrome out of 869 participants	40+ years	Metabolic syndrome: OR: 1.78 (1.02, 3.10)	1st vs. 4th quartile	n.s.
4 2009	Bronson-Castain KW Clinical study	Observational study	103 patients with type 2 DM	13-21 years	DM: Yes vs. No	n.s.	235.8 vs. 219.6 µm (p=0.03)
5 2009	Jeganathan VS Multiethnic Asian population study	Population-based, cross-sectional study	3404 participants with Fasting plasma glucose (FPG) tested		DM: Yes vs. control	143.6 vs. 145.3 µm (p=0.01)	n.s.
					FGP: each 1mmol/l ↑	n.s.	0.51 µm ↑ (p=0.006)

	Study	Study population and study design	Sample size and response rate	Age range	Metabolic factors	Retinal arteriolar caliber	Retinal arteriolar caliber
6 2008	Nguyen TT MESA	Population-based cross-sectional study	5976 patients	45-84 years	OGTT: control vs. IFG vs. DM	143.7 µm vs. 143.6 µm vs. 144.9 µm (p trend=0.008)	214.7 µm vs. 216.5 vs. 216.3 µm (p trend=0.02)
7 2007	Kifley A BMES	Population-based cross-sectional analysis	255 out of 3654 with diabetes, including 74 with		DM: Yes vs. No	193.5 vs. 190.2 µm (p=0.01)	n.s.
8 2006	Wong TY MESA	Americans Population-based cross-sectional study	5979	45-84 years	DM: Yes vs. No Serum glucose: each SD ↑	145.9 vs. 143.9 µm (p<0.001) 0.89 µm ↑ (p<0.001)	218.1 vs. 214.5 µm (p>0.001) 1.73 µm ↑ (p<0.001)
10 2004	Wong TY ARIC	American patients Population-based, cross-sectional study	11265 out of 15792	49-73 years	Metabolic syndrome: Yes vs. No	Generalized arteriolar Narrowing: OR: 1.23; (1.12,1.35)	Generalized venular dilatation: OR: 1.30; (1.18, 1.48)

Abbreviation: SINDI: the Singapore Indian Eye Study; MESA: Multi-Ethnic Study of Atherosclerosis; BMES: Blue Mountains Eye Study; ARIC: The Atherosclerosis Risk in Community.

Table 16. Associations between Metabolic Symptoms and Metabolic Syndromes and Retinal Vascular Tortuosity, Branching Angle and Fractal Dimension.

	Study	Study population and study design	Sample size and response rate	Age range	Metabolic factors	Retinal arteriolar characteristics	Retinal venular characteristics
1 2010	Sasongko MB SPDS	Sydney children and adolescents with type 1 diabetes Cross-sectional study	**Study 1**: 944 out of 1159 81.4%	12-20 years	A1C: ↑ DM duration: ↑	Tortuosity: ↑ (p=0.008) Branching angle: ↑ (p<0.001) optimality deviation: ↑ (p=0.018)	Tortuosity: n.s. Branching angle: n.s. optimality deviation: n.s.
2 2010	Yau JWY AusDiab Study	Population-based study	1577 patients with DM and IGT	>25 years	DM: OR: 1.64 (1.19, 2.27)	Fractal dimension: 3rd vs. 2nd tertile	
3 2007	Owen C	Case control study	17 Type 1 DM 36 Type 2 DM 60 controls	20-94 years	DM duration: Per 10 yrs ↑	Overall tortuosity: DM vs. non-DM patients 2.8%↓ (-4.3%, -1.3%)	

Abbreviation: SPDS: Sydney Pediatric Diabetes Study; AusDiab: the Australian Diabetes, Obesity and Lifestyle Study, ASCOT: An Anglo-Scandinavian Cardiac Outcomes Trial study.

1.3.2.2 Associations with Psychological Disorders and Neurological Diseases

Negative emotions such as anxiety and depression have been suggested to be associated with long-term incident cardiovascular disease and cerebrovascular disease, which is an important risk factor for the development of neurological disorders such as cognitive impairment, dementia and Alzheimer disease (AD).[108-111] Even there are structural cerebral microvascular alterations together with characteristics degenerative changes have been reported in dementia, pathophysiological changes on microcirculation in vivo among patients with psychological disorders and neurological diseases are still largely lacking.

In MESA, 423 participants aged 24 years and above who had the widest retinal arteriolar caliber (4^{th} quartile) had 2.81 times (1.38, 5.73) of getting peripheral neuropathy compared with those with the narrowest retinal arteriolar caliber (1^{st} quartile).[112] In the Cardiovascular Health Study (CHS), patients with focal arteriolar narrowing had 1.99 times (1.11, 3.56) in getting dementia.[111] A clinical study on early AD has shown that patients with early AD diagnosis had narrower retinal venular caliber than those without AD diagnosis (131.7 vs. 148.3 μm, p=0.01).[110] In BMES, 128 patients with migraine yet without aura had a 4.3 μm narrowing in retinal arteriolar caliber than those without migraine.[113] Cheung et al. also reported every 2 points increase in vital exhaustion assessment was associated with 1.19 times (1.03, 1.38) of getting generalized venular widening in ARIC White and African Americans.[114]

Table 17. Associations between Psychiatric and Psychological Conditions and Retinal Vascular Caliber

	Study	Study population and study design	Sample size and response rate	Age range	Neuropathy	Retinal arteriolar caliber	Retinal venular caliber
1 2010	Sabanayagam C MESA	Population-based, cross-sectional study	423 participants	24+ years	Peripheral neuropathy: OR: 2.81 (1.38, 5.73)	4th vs. 1st quartile	n.s.
2 2007	Baker ML CHS	Population-based, cross-sectional study	2211 with cognitive function analysis 1767 with dementia analysis	69 to 97 years	Dementia: OR: 1.99; (1.11, 3.56)	Focal arteriolar narrowing	n.s.
3 2007	Berisha F Clinical study	Patients with Early Alzheimer Disease	9 patients vs. 8 controls		Early Alzheimer Disease: Yes vs. No	n.s.	131.7 vs 148.3 μm (p=0.01)
4 2006	Liew G BMES	Population-based, cross sectional study	128 migraine without aura 182migraine with aura 1619 no migraine history		Migraine: Yes but no aura vs. No	4.3 μm↓ (0.5, 8.1)	n.s.
5 2009	Cheung N ARIC	White and African Americans Population-based, cross-sectional study	10365 out of 15792 65.6%	48-73 years	Vital exhaustion: each 2 points ↑	n.s.	Generalized venular widening: OR 1.19 (1.03, 1.38)

Abbreviation: MESA: Multi-Ethnic Study of Atherosclerosis; CHS: Cardiovascular Health Study; BMES: Blue Mountains Eye Study; ARIC: The Atherosclerosis Risk in Communities Study.

1.3.2.3 Associations with Food and Drugs Intake

There is increasing evidence showing dietary habit can lead to onset of a series of systematic diseases including hypertension, diabetes, cardiovascular diseases and stroke. Even though such relationships have been suggested, pathophysiological mechanisms underlying this potential association are unclear.[115] Researchers have started to investigate the impact of diet on retinal microcirculation. In BMES, fish or fish oil intake, glycemic index (GI) and low cereal fiber (CF) were assessed by validated food frequency questionnaires.[115, 116] The author found that persons consuming higher frequency of fish or fish oil had wider retinal arteriolar caliber (p trend=0.003) and wider retinal venular caliber (p=0.02).[116] Also, those consuming food in the highest GI tertile and lowest CF tertile were associated with retinal venular caliber widening (p trend<0.01), which resulted in a 5-fold increased risk of stroke death (hazard ratio: 5.06, 95% CI: 1.67, 15.22) after 10 years' follow up.[115] In ARIC, Kan et al. using intake of fiber instead of GI and CF yet similar results were found. Participants with the highest intake of fiber from all sources had a 1.05 μm increase in retinal arteriolar caliber (p trend=0.012) and a 1.11 μm decrease in retinal venular caliber (p trend=0.029), respectively.[117] From these two studies, retinal vascular abnormality was examined in subjects who had a less healthy diet, which might provide explanation on the relationship between dietary habit and vascular diseases.

Furthermore, the usage of medication are more and more studied to be associated with retinal vascular caliber changes, which might carry prognostic value in assessing drug effect on certain conditions or diseases.[43, 118-123] Wickremasing et al. studied 28

eyes with refractory diabetic macular edema (DME), 15 of which were treated with intravitreal triamcinolone (IVTA), which is a synthetic glucocorticoid to improve visual acuity and reduce macular thickening. Eye with IVTA treatment manifested a significant narrowing effect on both retinal arteriolar and venular caliber than those without IVTA, which showed improvement of the treatment.[119] Another eye disease nonexudative age-related macular degeneration (AMD) is known for a reduction in choroidal blood flow. One way to treat it is to use B-vitamin niacin, a known nicotinic acid which results in vasodilation and flushing. Thus, Barakat et al. examined 12 patients with AMD with niacin treatment or placebo and found that niacin produced vasodilatation of retinal arterioles.[123] Melistina et al. applied viagra on patients instead of niacin, and he found that the drug effect resulted in retinal venular widening instead of retinal arteriolar vasodilatation, which also showed drug effect.[120] In BDES, Wong et al. found that patients with beta-blocker eye drops or other type of eye drops for glaucoma treatment had both narrower retinal arteriolar and venular caliber.[118] For patients with hypertension, regular aspirin usage showed an increase of width in both retinal arterioles and venules.[121] For patients with premenopausal symptoms, those ever undertaking hormone replacement therapy (HRT) were found to be associated with narrowing in retinal arteriolar caliber and/or narrowing in retinal venular caliber.[43, 122] Leung et al. also studied the cocaine use in 68 African American HIV patients and those cocaine users had a 8.22 μm increase ($p=0.024$) and a 12.10 μm increase ($p=0.026$) in retinal arteriolar caliber and retinal venular caliber, respectively. The interesting finding might imply a potentially pathogenic effect of chronic cocaine use on dopamine receptors in retinal microcirculation. [124]

Table 18. Associations between Food and Drugs Intake and Retinal Vascular Caliber

	Study	Study population and study design	Sample size and response rate	Age range	Food and drugs intake	Retinal arteriolar caliber	Retinal venular caliber
1 2008	Kaushik S BMES	Population-based cohort study follow-up of 13 years	2683 out of 3654 73.0% 95 died from stroke	≥49 years	consuming any fish or oily fish: ↑	↑ (p=0.002)	↓ (p=0.02)
2 2007	Kan HD ARIC	White and African Americans Population-based, cross-sectional study	10659 out of 12887 82.7%	45-64 years	Fiber intake from all sources: 4th vs. 1st Qt	1.05 μm ↑ (p trend =0.012)	1.11 μm ↓ (p trend=0.029)
3 2008	Wickremasing SS Sydney hospital	Prospective randomized controlled trial with 2 months' follow up	28 eyes 15 (IVTA)	61.8 years	IVTA: Treated vs. Baseline	140.0 vs. 147.8 μm (p=0.047)	198.1 vs. 219.5 μm (p=0.039)
4 2008	Leung IYF	Clinical observational study	68 African Americans HIV patients	25-54 years	Cocaine: Users vs. Non- users	8.22 μm ↑ (p=0.024)	12.10 μm↑ (p=0.026)
5 2006	Barakat MR	Double-blind, randomized, placebo-controlled, crossover trial	12 AMD patients	72±7 years	Niacin: Yes vs. placebo 30 mins 90 mins	4.3 vs. -1.0% (p<0.05) 3.1 vs. -2.6% (p<0.05)	n.s. n.s.

	Study	Study population and study design	Sample size and response rate	Age range	Food and drugs intake	Retinal arteriolar caliber	Retinal venular caliber
6 2006	Metelitsina TI	Double-masked, randomized, placebo-controlled, crossover study	14 male patients, one eye with typical non-exudative AMD	75±7 years	<u>Viagra:</u> Yes vs. placebo 90 mins	n.s.	244 vs. 233 µm (p=0.0004)
					180 mins	n.s.	248 vs. 235 µm (p<0.0001)
					300 mins	n.s.	239 vs. 226 µm (p<0.0001)
7 2005	Wong TY BDES	All women participants Population based cross-sectional study	2469 10.5% current users of ERT 7.4% past users of ERT	43-84 years	<u>ERT:</u> Current vs. past vs. non users	167.6 vs. 170.8 vs. 170.9 µm (p=0.01)	239.9 vs. 244.0 vs. 243.9 µm (p=0.02)
					<u>Duration of ERT:</u> 4th vs. 1st quartile	↓ (p trend=0.007)	n.s.
8 2005	Liew G BMES	Population-based cohort study	775 with regular aspirin use at baseline	49+ years	<u>Aspirin & antihypertensive combined:</u> Yes vs. Anti-hypertensive alone	195.3 vs. 191.8 µm (p<0.01)	n.s.
					<u>Regular aspirin</u>: Yes vs. No	194.4 vs. 189.6 µm (p<0.05)	226.6 vs. 222.6 µm (p<0.05)

	Study	Study population and study design	Sample size and response rate	Age range	Food and drugs intake	Retinal arteriolar caliber	Retinal venular caliber
9 2005	Wong TY BDES	Population-based, cross-sectional study	4608 patients	43-84 years	Beta-blocker eye drops for Glaucoma: Yes vs. No	167.0 vs. 170.8 µm (p=0.05)	238.5 vs. 245.6 µm (p=0.006)
					Glaucoma eye drops Yes vs. No	167.2 vs. 170.9 µm (P=0.03)	240.1 vs. 245.6 µm (P=0.01)
10 2004	Leung H BMES	Population-based, cross-sectional study Hormone replacement therapy (HRT) normally estrogen	1897 women 79 premenopausal 315 current HRT 224 past HRT 1279 never HRT	49+ years	HRT: Ever (current or past user) vs. never users	Generalized arteriolar narrowing OR=1.4 (1.0, 1.8)	n.s.

Abbreviation: BMES: the Blue Mountains Eye Study, ARIC: the Atherosclerosis Risk in Community; BDES: the Beaver Dam Eye Study.

1.3.2.4 Associations with Vascular Diseases

There is a wide range of vascular diseases including macrovascular and microvascular diseases, such as hypertension, atherosclerosis, cardiovascular disease and cerebrovascular disease. Also, architectural changes in vascular anatomy are considered as dysfunctional markers of peripheral vessels and special circulation in organs like eyes, heart and brain.

Numerous studies have reported that retinal microcirculation abnormality is associated with hypertension. In MESA, patients with uncontrolled hypertension or controlled hypertension had a significantly narrower retinal arteriolar caliber than healthy non-hypertensive controls (140.0 vs. 142.1 vs. 146.0 μm, p trend<0.0001).[59] In BMES, Wang et al. reported that patients with hypertension regardless of treatment effect (untreated/uncontrolled/controlled) had 1.5-2.1 (95% CI: 1.1, 2.7) times risk of generalized retinal arteriolar narrowing compared to those without hypertension.[125] Also, in the same population, Liew et al. reported patients with hypertension had a 0.01 Df smaller (p=0.02) in fractal dimension than those without hypertension.[35] In two clinical observational studies, hypertensive patients were suggested to have higher retinal venular tortuosity than control and higher total retinal vascular tortuosity than diabetic patients, respectively.[126, 127]

Retinal microcirculation has also been suggested to be associated with a series of peripheral vascular markers for function assessments. For example, Nguyen et al. reported the relationship between lower flow-mediated dilation (FMD) and retinal venular widening[128] and Pressler et al. reported the relationship between coarctation

repair and retinal arteriolar narrowing.[129] In MESA, each standard deviation decrease in large-artery compliance was associated with a 0.70 μm decrease (p=0.002) and a 1.45 μm increase (p=0.001) in retinal arteriolar and venular caliber, respectively.[130] In the same population, patients classified into the lowest aortic distensibility had 1.72 times risk of generalized arteriolar narrowing compared with those classified into the highest aortic distensibility.[131] In Hoorn and MCRS, increased carotid intima-media thickness and ipsilateral severe carotid disease were both significantly associated with retinal venular widening.[132, 133] From all these studies, we can speculate that retinal vascular caliber abnormalities (retinal arteriolar narrowing and retinal venular widening) are related to adverse functioning of peripheral vessels.

Cardiovascular disease has been widely reported to be associated with retinal microcirculation partly explained by their relationships with hypertension. In ARIC, patients with left ventricular hypertrophy (LVH) had 1.43 times risk of generalized arteriolar narrowing compared with those without LVH.[16] In MESA, each standard deviation decrease in retinal arteriolar caliber was associated with 2.06 times risk of concentric remodeling.[134] Similarly in measuring cardiovascular functioning, indexes like lower hyperemic blood flow and lower perfusion reserve were suggested to be significantly associated with retinal arteriolar narrowing.[135] As for heart diseases, higher total retinal vascular tortuosity and lower arteriolar diameters at bifurcation were suggested in cyanotic congenital heart disease and ischemic heart disease by Tsui et al.[136] and Witt et al.,[17] respectively.

The retinal and cerebral microcirculation share similar anatomical, physiological, and embryological characteristics. Disease of small arteries and arterioles has been

hypothesized to be a major risk for stroke, and may also be the underlying cause of magnetic resonance imaging (MRI)-defined white matter lesions.[1] Thus, there is a strong biological rationale for studying retinal vessels to understand cerebral microvascular diseases. Stroke is the most widely studied cerebrovascular disease. Data from different population-based Caucasian studies have shown that lacunar stroke subtype was associated with retinal venular widening and higher fractal dimension by Doubal et al. and Cheung et al., respectively.[137, 138] Also, Doubal et al. compared lacunar stroke and cortical stroke and found smaller monofractal dimension and multifractal dimension were both associated with lacunar stroke rather than cortical stroke.[137] Data from ARIC showed that patients with cerebral infarcts occurrence was more likely to be associated with focal arteriolar narrowing.[13] As for cerebrovascular pathological changes, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) was associated with retinal venular widening,[139] smaller AVR[140] and smaller fractal dimension.[141]

Furthermore, ocular circulation due to certain medical condition or disease was also investigated its relationship with retinal microcirculation, such as branch retinal vein occlusion (BRVO) and macular telangiectasia (MT). Youm et al. investigated 10890 participants and found that patients with BRVO had a 5.6 μm (p=0.004) narrower retinal arteriolar caliber and a 7.6 μm (p=0.001) wider retinal venular caliber than those without BRVO, respectively.[142] In a multi-center case-control study, Tikellis et al. found each standard deviation increase in retinal arteriolar caliber and retinal venular caliber was associated with 2.18 times (95% CI: 1.50, 3.18) and 1.87 times (95% CI: 1.31, 2.67) of getting MT, respectively.[143]

Table 19. Associations between Vascular Risk Factors and Retinal Vascular Caliber

	Study	Study population and study design	Sample size and response rate	Age range	Vascular risk factors	Retinal arteriolar caliber	Retinal venular caliber
1 2012	Youm DJ	Hospital-based cross-sectional study	10890 participants BRVO: Branch Retinal Vein Occlusion	50+ years	BRVO: Yes vs. No	142.6 vs. 148.2 µm (p=0.004)	211.1 vs. 203.5µm (p=0.001)
2 2010	Nguyen TT MESA	Multi-ethnic Americans Population-based cross-sectional study	2851 FMD: Flow-mediated dilation	45-84 years	FMD: 0.25 ↓ (-0.36, -0.13) (p<0.01)	n.s.	each SD ↑
3 2010	Pressler A Clinical study	Case-control, cross-sectional	34 patients after coarctation repair 34 non-hypertensive controls	23-58 years	Coarctation repair: Yes vs. No	182 vs. 197 µm (p<0.001).	n.s.
4 2010	Baker ML MCRS	Patients with acute ischemic stroke recruited from Melbourne, Sydney and Singapore	1565 1360 with acute stroke 86.9%	19-94 years	Subcortical atrophy in non DM: OR: 1.9 (1.0, 3.8)	n.s.	Generalized retinal venular widening: Yes. vs. No.
5 2009	Tikellis G Multi-center Macular Telangiectasia Project	Patients with MT Case-control study	55 cases MT 170 age and DM status matched MT: Macular Telangiectasia	37-91 years	MT: OR: 2.18 (1.50, 3.18) MT: OR:1.87 (1.31, 2.67)	each SD ↑ n.s.	n.s. each SD ↑

	Study	Study population and study design	Sample size and response rate	Age range	Vascular risk factors	Retinal arteriolar caliber	Retinal venular caliber
6 2009	Doubal FN Clinical study	Patients with lacunar ischemic stroke and cortical stroke controls Cross-sectional study	212 105 lacunar 107 cortical	Mean age of 68 years	<u>Lacunar stroke subtype:</u> Yes vs. No	n.s.	1.33 µm ↑ (p=0.03)
7 2009	De Silva DA MCRS	Patients with acute ischemic stroke recruited from Melbourne, Sydney and Singapore	1029 7% with severe extracranial carotid disease	41-89 years	<u>Severe ipsilateral carotid disease:</u> OR: 3.81 (1.80, 8.07)	n.s.	4^{th} vs. 1^{st}. quartile
8 2009	Jeganathan V MESA	Population-based, cross-sectional study	3749	24+ yrs	<u>HTN</u>: uncontrolled vs. controlled vs. non	140.0 vs. 142.1 vs. 146.0 µm (p<0.0001)	n.s.
9 2008	Tikellis G ARIC study	African-American participants Population-based, cross-sectional	1439 LVH: left ventricular hypertrophy	45-64 years	<u>LVH:</u> Yes vs. No	<u>Generalized arteriolar narrowing</u> OR: 1.43 (1.08, 1.89)	n.s.
10 2007	Wang L MESA	Multi-ethnic Americans Population-based cross-sectional study	212 free of CVD 98 free of coronary artery calcification	45-84 years	<u>Hyperemic blood flow:</u> each SD ↓	↓ (p trend=0.006)	n.s.
					<u>Perfusion reserve:</u> each SD↓	↓ (p trend=0.01)	n.s.

	Study	Study population and study design	Sample size and response rate	Age range	Vascular risk factors	Retinal arteriolar caliber	Retinal venular caliber
11 2007	Cheung N MESA	Multi-ethnic Americans Population-based cross-sectional study	5731 free of clinical CVD	45-84 years	Large-artery compliance: each SD ↓	0.70 µm ↓ (p=0.002)	1.45 µm ↑ (p=0.001)
12 2007	Cheung N MESA	Multi-ethnic Americans Population-based cross-sectional study	3425 free of clinical CVD	45-84 years	Aortic distensibility: 1st vs.4th quartile	Generalized arteriolar narrowing OR: 1.72 (1.15,2.58)	n.s.
13 2007	Cheung N MESA	Multi-ethnic Americans Population-based cross-sectional study	4953 free of clinical CVD Concentric remodeling is defined as highest quintile of M/V ratio	45-85 years	Concentric remodeling: OR: 2.06 (1.57, 2.70)	each SD ↓	n.s.
14 2006	Cooper LS ARIC	Population-based, cross-sectional study	1684	55-74 years	Cerebral infarcts: OR: 1.89 (1.22, 2.92)	Focal arteriolar narrowing Yes vs. No	n.s.
15 2006	Van Hecke Hoorn Study	Caucasian Population-based, cross-sectional study	256 out of 2484 70 normal 69 IGT 109 Type 2 DM IMT: intima-media thickness	60-85 years	Carotid IMT: 0.14 mm ↑ (0.005, 0.25)	n.s.	each SD ↑

	Study	Study population and study design	Sample size and response rate	Age range	Vascular risk factors	Retinal arteriolar caliber	Retinal venular caliber
16 2005	Roine S Clinical study	Patients with(CADASIL) Cross-sectional study	38 CADASIL 16 healthy controls	20-64 years	CADASIL patients	↓ AVR (p<0.001)	
17 2003	Wang JJ BMES	Population-based, cross-sectional study	3654 HTN: Hypertension Reference group: normotensive subjects	≥49 years	HTN: controlled vs. uncontrolled vs. Untreated	Generalized arteriolar narrowing OR: 1.5 (1.1, 1.9) vs, OR: 2.1 (1.6, 2.7) vs. OR: 2.1 (1.6, 2.7)	n.s.

Abbreviation: MESA: Multi-Ethnic Study of Atherosclerosis; MCRS: The Multi-Centre Retinal Stroke (MCRS) Study; ARIC: the Atherosclerosis Study in Community; BMES: the Blue Mountains Eye Study.

Table 20. Associations of Vascular Risk Factors and Retinal Vascular Tortuosity, Branching Angle and Fractal Dimension

	Study	Study population and study design	Sample size and response rate	Age range	Vascular risk factors	Retinal arteriolar characteristics	Retinal venular characteristics
1 2011	Cavallari M	Clinical study	10 patients CADASIL: Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy		CADASIL: Yes. vs. No	Fractal dimension: 1.42 vs. 1.50 (pair-t-test p=0.002)	
2 2010	Doubal FN Clinical study	Hospital based, case-control study	166 out of 183 eligible 86 with lacunar stroke 80 with cortical stroke	Mean age 67.3 years	Lacunar stroke subtype vs. Cortical stroke	Monofractal Dimension(Dbox): ↓0.01 (p<0.001) Multifractal dimension (DO): ↓0.001 (p=0.002)	
3 2010	Cheung N Multi-center Retina Stroke Study	Patients with acute ischemic stroke Cross-sectional study	392 with lacunar stroke	Mean age 67.2 years	Lacunar stroke OR: 4.27 (1.49, 12.17)	Fractal dimension: 4th vs. 1st quartile	
4 2009	Tsui I	Hospital-based, clinical study	4 patients with cyanotic congenital heart disease	27-47 years	Cyanotic congenial heart disease: Yes vs. No	Retinal vascular tortuosity: ↑	
5 2008	Liew G BMES	Population-based cohort study	300 random samples 100 HTN 100 DM 100 without HTN	49-97 years	HTN: Yes vs. No	Fractal dimension: 0.01 ↓ (p=0.02)	

	Study	Study population and study design	Sample size and response rate	Age range	Vascular risk factors	Retinal arteriolar characteristics	Retinal venular characteristics
6 2006	Hughes AD	Clinical observational study	20 patients with essential HTN 20 with malignant phase HTN 20 normotensive controls		HTN: Malignant vs. controls	n.s.	Retinal Venular Tortuosity: ↑
7 2006	Witt N BDES	Population-based, cross-sectional study	4926 out of 5924 (83%) 417 incident CVD death 173 incident CVD death	43-86 years	IHD: Yes vs. No	Suboptimal arteriolar diameters at bifurcation: ↓ (p=0.02)	n.s.
8 2005	Zhou P	Clinical observational study	Patients with HTN and DM		HTN vs. DM	Total retinal tortuosity: ↑	

Abbreviation: SPDS: Sydney Pediatric Diabetes Study; BMES: the Blue Mountains Eye Study; BDES: the Beaver Dam Eye Study.

1.3.3 Birth Parameters with Retinal Microvasculature

A large body of literature shows that susceptibility to cardiovascular disease may have etiological origins in utero and in infancy.[144-146] In epidemiological data reported by quite a few population-based studies, it was suggested that poorer early life growth such as lower birth weight and smaller head circumference was associated with higher blood pressure in childhood[146-148] and risk of cardiovascular disease in adulthood.[149] Based on these findings, researchers have hypothesized that early growth may affect small vessel structure and function. Thus, more and more recent studies started to investigate the association between birth parameters and retinal microcirculation, which may reveal the physiological mechanisms between fetal growth and development of adult disease.

It has been repeatedly reported that smaller birth parameters were associated with retinal arteriolar narrowing, smaller fractal dimension or retinal venular widening both in children and adults.[150-154] Each SD decrease in birth weight was associated with a 1.28 μm narrowing in retinal arteriolar caliber and a 1.49 μm in retinal venular caliber.[151-154] In SCES, Mitchell found a 3.73 μm narrower retinal arteriolar caliber among children with low birth weight (<2500 g) than those with normal birth weight.[151] Similar magnitude was found in retinal arteriolar narrowing with each SD decrease in other birth parameters such as birth length, head circumference.[150, 151, 153] For gestational age, premature babies had a 3.43 μm narrower retinal arteriolar caliber than full-term babies.[151] The changes in retinal arteriolar caliber might be a sign of endothelial dysfunction which is possible early predisposition of any CVD risk later in life.

Table 21. Associations between Birth Parameters and Retinal Vascular Parameters

	Study	Study population and study design	Sample size and response rate	Age range	Birth parameters	Retinal arteriolar characteristics	Retinal venular characteristics
1 2010	Gopinath B SCES	Australian adolescents Population-based, cross-sectional study	2353 out of 3144 75.3%	Mean age 12.7 years	Birth weight: 1st vs. 4th quartile	CRAE: 2.5 µm ↓ (p trend=0.001)	n.s.
					Birth length: ↓	CRAE: ↓	n.s.
					Head circumference: ↓	CRAE: ↓	n.s.
					Head circumference: ↓ (p trend =0.03)	Total fractal dimension: ↓	
2 2009	Sun C Australian Twins Eye Study	Twin-based, cross-sectional study	266 twins (49 monozygotic and 84 dizygotic pairs)	5-90 years	Head circumference: each 2 cm ↓	CRAE: 2.55 µm ↓ (-4.92, -0.18) (p=0.04)	n.s.
					Birth length: each 5 cm ↓	CRAE: 7.27 µm ↓ (-11.54, -3.01) (p<0.001)	n.s.
3 2008	Liew G ARIC	White and African Americans Population-based, cross-sectional study	3800 68.2%	51-72 years	Birth weight: each 1 kg ↑	CRAE: 2.4 µm ↓ (-1.3, -3.5) (p<0.001)	--

	Study	Study population and study design	Sample size and response rate	Age range	Birth parameters	Retinal arteriolar characteristics	Retinal venular characteristics
4 2008	Mitchell P SCES	Australian adolescents Population-based, cross-sectional study	1369 out of 3144 78.7%	Mean age 12.7 years	Birth weight: each SD ↓	CRAE: 1.28 μm ↓ (-2.23, -0.32) (p trend=0.01)	n.s.
					Birth weight: Low vs. normal	CRAE: 3.73 μm ↓ (-7.09, -0.38) (p=0.03)	n.s.
					Birth length: each SD ↓	CRAE: 1.0 μm ↓ (-1.89, -0.12) (p=0.03)	n.s.
					Head Circumference: each SD ↓	CRAE: 1.24 μm ↓ (-2.09, -0.38) (p=0.006)	n.s.
					Gestational age: Premature vs. Full-term	CRAE: 3.43 μm ↓ (-5.95, -0.91) (p=0.009)	n.s.
5 2008	Cheung N SCORM	School-based, cross-sectional study	561 out of 768 children 73.0%	7-9 years	Birth weight: Each SD ↓ (SD=453 g)	n.s.	CRVE: 1.49 μm ↑ (0.18, 2.80) (p=0.03)

Abbreviation: SCES: Sydney Children Eye Study; ARIC: the Atherosclerosis Risk in Community; SCORM: the Singapore Cohort Study of the Risk Factors of Myopia.

1.4 Retinal Vascular Changes and Long-Term Morbidity and Mortality of Systemic Diseases

Numerous longitudinal studies have reported that retinal microvascular abnormalities are related to both present and future occurrence of systemic diseases such as hypertension, diabetes, cardiovascular diseases and stroke. In order to better understand the predictive value of retinal microvasculature on diseases onsets, we also looked into these associations and selected diseases were presented in **Table 22-25**.

Table 22 showed the association between retinal vascular parameters and incident hypertension. Each SD decrease in retinal arteriolar caliber and retinal venular caliber was associated with 1.2-1.39 (95% CI: 1.02, 1.55) times and 1.17-1.18 (95% CI: 1.02, 1.40) times in getting incident hypertension after 3-7 years' follow up in MESA and the Rotterdam Study.[17, 155] Similarly, participants with the lowest quartile of retinal arteriolar caliber and AVR resulted in 1.53-1.82 times (95% CI: 1.08, 2.40) in getting incidence of hypertension than those with the highest quartile or quintile of values after 3-10 years' follow up in Funagata study, BDES and ARIC.[156-158] In BMES, participants classified into generalized retinal arteriolar narrowing had 2.6 times (95% CI: 1.7, 3.6) of getting severe hypertension after mean follow-up of 5 years.[159]

The association between retinal microvasculature and incidence of metabolic diseases and related complications were also widely studied. Narrower retinal arteriolar caliber, wider retinal venular caliber or smaller AVR were associated with future impaired fasting glucose and even diabetes in multiple population-based

studies as BMES, AusDiab Study, the Rotterdam study, BDES and ARIC.[30, 160-163] Among diabetic patients without diabetic retinopathy at baseline, retinal venular caliber widening was a significant marker for predicting diabetic retinopathy (DR) and even progression-proliferative DR in 3-7 years' time.[164-167] Compared to retinal venular widening, retinal arteriolar widening had a less striking prediction on DR and its subtypes. Retinal vascular parameters such as retinal arteriolar simple tortuosity and fractal dimension were recently studied on their relationships with incident DR.[168, 169] In a prospective cohort study of children and adolescent with type 1 diabetes, Benitez-Auirre et al. and Cheung et al. found that greater value of these two parameters mentioned earlier was associated with higher risk win getting DR in 3.8 years' time.[168, 169]

The associations between retinal vascular parameters and incidence of vascular diseases morbidity and mortality were widely investigated, the latter of which varied from chronic kidney disease, heart disease, stroke and even eye disease, and mortality of major vascular diseases and even AIDS (**Table 24**). In MESA, the lowest quartile of retinal arteriolar caliber was associated with a hazards ratio of 1.78 (95% CI: 1.01, 3.15) in getting future chronic kidney disease stage 3 in a median 4.8 years' follow-up.[170] In WESDR, the highest quartile of retinal venular caliber was associated with incident gross proteinuria (HR: 1.53; 95% CI: 1.19, 1.97) and incident renal insufficiency (HR: 1.51; 95% CI: 1.05, 2.17) after 16 years' follow-up.[48, 171] Also, in type 1 DM adolescents, other changes in parameters like wider retinal venular length-to-diameter ratio (LDR) and smaller simple tortuosity were also found to be associated with 1.55 and 1.69 times in getting incident renal dysfunction,

respectively.[172] Furthermore, retinal arteriolar narrowing and/or retinal venular widening were associated with future amputation, coronary artery disease (CAD) and all types of coronary heart disease (CHD) such as acute myocardial infarction among children and adults across different ethnicities.[48, 173-176] For example, each standard deviation decrease in retinal arteriolar caliber and each standard deviation increase in retinal venular caliber was associated with a hazard ratio of 1.27 (95% CI: 1.08, 1.50) and 1.31 (95% CI: 1.10, 1.56) in incidence of CHD after 8.8 years' follow-up, respectively.[176] Same directions were found between retinal arteriolar narrowing and/or retinal venular widening and CHD-mortality in BDES and BMES.[177, 178] In BMES, suboptimal fractal dimension (the highest and the lowest quartile) was also found to be associated with CHD mortality in patients aged 70 years and below with a significant hazard ratio (1.30 and 1.89).[179] For cerebrovascular diseases, stroke and stroke-related mortality were repeatedly suggested to be predicted by retinal arteriolar narrowing and retinal venular widening, due to the similar share in anatomical, physiological and embryological characteristics between retinal circulation and cerebrovascular circulation.[48, 174, 177] Incidence of stroke and its subtypes such as intracerebral hemorrhages, cerebral infarctions and lacunar infarcts have been associated with retinal venular caliber in the Rotterdam study[119] and CHS[174] and with retinal arteriolar narrowing in a prospective cohort reported by Kwa et al.,[180] while 10-22 years' stroke mortality was associated with either retinal arteriolar narrowing or retinal venular caliber in WESDR,[48] BDES and BMES.[177] For other cerebral diseases, retinal venular caliber was associated with incident vascular dementia, periventricular and subcortical white matter lesion (WML) progression in the

Rotterdam study[181, 182] while retinal arteriolar narrowing was associated with incident WML in a prospective cohort reported by Kwa et al..[180] Retinal arteriolar narrowing and smaller AVR were also suggested to be associated with a 18% increase in all causes-mortality in WESDR[48] and a 12% increase AIDS-related mortality in a longitudinal study of the ocular complications of AIDS (LSOCA).[183 184] Since the association has been established between ocular diseases and retinal vascular parameters in many population-based and cross-sectional studies, the prediction of abnormal retinal vascular signs were also being investigated yet the findings weren't consistent. The longitudinal data were shown in Table 30. In BMES, the lowest quartile of retinal arteriolar caliber was associated with lower risk in incidence of nuclear cataract (OR: 0.62; 95% CI: 0.42, 0.92) and with higher risk in incidence of Posterior Subcapsular Cataract (PSC) (OR: 2.40; 95% CI: 1.34, 4.29) and cataract surgery (OR: 1.52; 95% CI: 1.06, 2.17) after 5-10 years' follow-up.[185] However, any change in retinal arteriolar caliber or retinal venular caliber was not associated with visual function in AIDS patients[186] or associated with early-stage age-related maculopathy (ARM) in BMES[187] or associated with age-related macular degeneration (AMD)[184] and open-angle glaucoma (OAG) in the Rotterdam study[184] after at least 5 years' follow-up.

Table 22. Associations between Retinal Vascular Caliber and Incidence of Hypertension

	Study	Study population and study design	Sample size and response rate	Age range/ yrs of follow up	Incidence of Hypertension	Retinal arteriolar caliber	Retinal venular caliber
1 2010	Kawasaki T The Funagata Study	Japanese population-based cohort study	581 patients 193 developed hypertension	35+ years 5-years follow up	OR: 1.53 (1.08, 2.18)	1st vs. 4th quartile	n.s.
2 2009	Kawasaki R MESA	Multi-ethnic Americans; Population-based cohort study	2583 out of 6237 448 incident hypertension	45-87 years Median follow up of 3.2±0.5 years	OR: 1.2 (1.02, 1.42)	Per SD ↓	
					OR: 1.18 (1.02, 1.37)		Per SD ↓
3 2005	Ikram MK Rotterdam study	European Caucasians with normal glucose tolerance test Population-based prospective longitudinal study	1900 out of 4940 739 normal BP 1161 preHTN	≥55 years Mean follow-up 6-7 years	OR: 1.38 (1.23, 1.55)	Per SD ↓	
					OR: 1.17 (1.04, 1.32)		Per SD ↓
					OR: 1.24 (1.10, 1.40)	Per SD ↓ in AVR	
4 2004	Smith W BMES	Population-based cohort study	1319 out of 2335 normotensive or mild (grade 1) hypertension at baseline 75.1% 390 severe HTN	≥49 years Mean follow-up of 5 years	Severe HTN OR: 2.6 (1.7, 3.9)	Generalized retinal arteriolar narrowing: Yes vs. No	n.s.

	Study	Study population and study design	Sample size and response rate	Age range/ yrs of follow up	Incidence of Hypertension	Retinal arteriolar caliber	Retinal venular caliber
5 2004	Wong TY BDES	Patients living in Wisconsin Prospective cohort study	2451 normotensive 721 hypertension	43 -84 years Follow-up of 10 years	OR: 1.82 (1.39, 2.40)	1^{st} vs. 4^{th} quartile in AVR	
6 2004	Wong TY ARIC	White and African Americans Population-based cohort study	5628 811 incident HTN	49-73 years Mean follow up of 3 years	OR: 1.69 (1.21, 2.18)	1^{st} vs. 4^{th} quartile in ARV	

Abbreviation: MESA: The Multi-Ethnic Study of Atherosclerosis; BMES: the Blue Mountains Eye Study; BDES: the Beaver Dam Eye Study; ARIC: the Atherosclerosis Risk in Community.

Table 23. Associations between Retinal Vascular Parameters and Incidence of Metabolic Diseases

	Study	Study population and study design	Sample size and response rate	Age range/ Yrs of follow-up	Incidence of Metabolic Diseases	Retinal arteriolar characteristics	Retinal venular characteristics
1 2008	Kifley A BMES	Population-based, cohort study	3368 patients	49+ years 5-year follow up	IFG: OR: 1.53 (1.11, 2.12)	n.s.	CRVE: 4th vs. 1st quartile
2 2008	Nguyen TT AusDiab Study	Australian whites Population-based cohort study	803 without diabetes at baseline 108 cases	Mean follow up of 5 years	DM: OR: 2.21 (1.02,4.80)	CRAE: 1st vs. 4th quartile	n.s.
3 2006	Ikram MK Rotterdam study	European Caucasians with normal glucose tolerance test Population-based prospective longitudinal study	2309 out of 4940	≥55 years Mean follow-up 6-7 years	IFG: OR: 1.13 (1.00-1.29) DM: OR: 1.09 (0.90, 1.33)	n.s. n.s.	CRVE: each SD ↑ CRVE: each SD ↑
4 2005	Wong TY BDES	Patients living in Wisconsin Prospective cohort study	3251 249 incident diabetes	43-86 years	DM RR: 1.53 (1.03, 2.27)	1st vs. 4th quartile in AVR	
5 2002	Wong TY ARIC	American Caucasians Population-based, Cohort study	7993 3.5 years' follow up	49-73 years	Incident: OR: 1.71 (1.13, 2.57)	1st vs. 4th quartile in AVR	

	Study	Study population and study design	Sample size and response rate	Age range/ Yrs of follow-up	Incidence of Metabolic Diseases	Retinal arteriolar characteristics	Retinal venular characteristics
6 2011	Benitez-Aguirre P Clinical research	Prospective cohort study Hospital based	736 adolescence with Type 1 DM 287 developed retinopathy	12-20 years Median 3.8 years	DR: OR: 1.5 (1.0, 2.2)	Simple tortuosity: 4th vs. 1st quartile	n.s.
7 2009	Cheung N Prospective cohort study of children and adolescents with type 1 diabetes	Prospective cohort study Hospital based	729 adolescence with Type 1 DM 137 developed retinopathy	12-20 years Median 3.8 years	DR: OR: 3.92 (2.02, 7.61) DR: OR: 1.37 (1.21, 1.56)	Fractal dimension: 4th vs. 1st quartile Fractal dimension: Each 0.01 ↑	
8 2011	Roy MS	Hospital based	468 African Americans with type 1 DM	<30 years 6 years follow up Progression-proliferative DR (P-PDR)	P-PDR: OR: 4.63 (2.02, 10.63) P-PDR with high risk characteristics OR: 4.62 (1.61,13.29)	n.s. n.s.	CRVE: 4th vs. 1st quartile CRVE: 4th vs. 1st quartile
9 2008	Roger SL AusDiab study	Australian whites Population-based cohort study	250 with diabetes 455 with IFG 27 developed DR	Mean follow up of 5 years	DR: OR: 4.79 (1.57, 14.58)	n.s.	CRVE: 4th vs. 1st quartile

	Study	Study population and study design	Sample size and response rate	Age range/ Yrs of follow-up	Incidence of Metabolic Diseases	Retinal arteriolar characteristics	Retinal venular characteristics
10 2008	Roger SL AusDiab study	Australian whites Population-based cohort study	250 with diabetes 455 with impaired fasting glucose or impaired glucose tolerance 27 incident retinopathy	Mean follow up of 5 years	DR: OR: 4.79 (1.57, 14.58)	n.s.	CRVE: 4th vs. 1st quartile
11 2006	Alibrahim E Clinical study	Australian children and adolescents with type 1 diabetes Hospital-based case-control study with prospective outcome	668 baseline 172 incident retinopathy 180 age, gender-matched control	12-20 years Mean follow-up 3.1-3.6 years	DR: OR: 1.44 (1.11. 1.86)	CRAE: each SD ↑	n.s.
12 2004	Klein R WESDR	American whites Population-based, cross-sectional study	996 at baseline with DM 891 4 years' follow up	≥30 years 4-year's follow-up progression of DR (Prog-DR) Proliferative DR (Proli-DR)	Prog-DR: HR: 2.04 (1.20, 3.47) Prog-DR HR: 2.33 (1.37, 3.95) Proli-DR: HR: 4.28 (1.50, 2.19)	CRAE: 4th vs. 1st quartile n.s.	n.s. CRVE: 4th vs. 1st quartile

Abbreviation: AusDiab study: Australian diabetes, obesity and lifestyle study; BMES: the Blue Mountains Eye Study; BDES: the Beaver Dam Eye Study; WESDR: the Wisconsin Epidemiologic Study of Diabetic Retinopathy.

Table 24. Association between Retinal Vascular Parameters and Incidence of Vascular Diseases Morbidity and Mortality

	Study	Study population and study design	Sample size/ response rate	Age range/ Yrs of follow-up	Incidence of morbidity and mortality	Retinal arteriolar characteristics	Retinal venular characteristics
1 2011	Gangaputra S LSOCA	Caucasian and African American AIDS patients Longitudinal observational cohort study	1250 without ocular opportunistic infections at baseline; 304 deaths	38-48 years Mean follow-up 6.8 years	AIDS-related Mortality: 12% ↑ (p=0.02)	AVR: each quartile ↓	
2 2011	Yau JWY MESA	Multi-ethnic Americans; Population-based cohort study	5979 out of 6814 67.4% 232 incident CKD stage 3 cases	45-84 years Median follow up of 4.8 years	CKD stage 3 In Caucasian: HR: 1.78 (1.01, 3.15)	CRAE: 1st vs. 4th quartile	n.s.
3 2004	Wong TY WESDR	Americans with type 1 diabetes Population-based, cross-sectional study	903 at second examination 557 incident gross proteinuria and renal insufficiency	≥30 years Mean follow up 16 years	Gross proteinuria: HR: 1.53 (1.19, 1.97) Renal insufficiency: HR: 1.51 (1.05, 2.17)	n.s.	CRVE: 4th vs. 1st quartile
4 2007	Klein R WESDR	American whites Population-based, cross-sectional study	1370 with type 2 DM at baseline	30+ years 10 years, 14 years and 22 years' follow-up	10 years' proteinuria: HR: 2.08 (1.47, 2.94)	n.s.	CRVE: 4th vs. 1st quartile

	Study	Study population and study design	Sample size/ response rate	Age range/ Yrs of follow-up	Incidence of morbidity and mortality	Retinal arteriolar characteristics	Retinal venular characteristics
5 2012	Benitez-Aguirre P Clinical research	Prospective cohort study Hospital based	511 adolescents with Type 1 DM 174 developed renal dysfunction	12-20 years Median 3.7 years' follow-up	<u>Renal dysfunction:</u> OR: 1.69 (1.17, 2.44) <u>Renal dysfunction:</u> OR: 1.55 (1.08, 2.22)	n.s. n.s.	<u>Venular LDR</u>: 4th vs. 1-3rd quartile <u>Venular ST</u>: 1st vs. 2-4th quartile
6 2011	Liew G BMES	Population-based, cohort study	3303 468 CHD deaths	≥49 years Follow-up 14 years	<u>CHD-mortality in all patients:</u> HR: 1.5 <u>CHD-mortality in pt ≤70 years:</u> HR: 1.30-1.89 (1.25, 2.84)	<u>Fractal dimension:</u> 1st and 4th quartile (suboptimal) vs. 2nd and 3rd quartile	
7 2008	Miller RG EDC	Children <18 years at diagnosis without history of laser photocoagulation Prospective study of childhood-onset T1D	448 out of 658 68.1% 80 Coronary artery disease (CAD) 368 non cases	Median follow-up of 18 years	<u>CAD:</u> HR: 1.42 (1.04, 1.96)	<u>CRAE:</u> 1st vs. 4th quartile	<u>CRVE:</u> n.s.

	Study	Study population and study design	Sample size/ response rate	Age range/ Yrs of follow-up	Incidence of morbidity and mortality	Retinal arteriolar characteristics	Retinal venular characteristics
8 2008	McGeechan K ARIC	White and African Americans Population-based cohort study	9155 out of 15792 700 incident coronary heart disease (CHD)	45-64 years Mean follow up of 8.8 years	CHD: HR: 1.27 (1.08,1.50) CHD: HR: 1.31 (1.10,1.56)	CRAE: each SD ↓	CRVE: each SD ↑
9 2007	Wang JJ BDES and BMES	Pooled data analysis	7497 out of 8550 (88%) 653 CHD death 299 Stroke death	43-97years Mean follow up more than 10-12 years	CHD-mortality: HR: 1.34 (1.11, 1.62) CHD-mortality: HR: 1.24 (1.02, 1.52)	CRAE: 1st vs. 5th quintile	CRVE: 5th vs. 1st quintile
10 2007	Klein R WESDR	American whites Younger group Population-based, cross-sectional study	1370 with type 2 DM at baseline	30+ years 10 years, 14 years and 22 years' follow-up	14 years' amputation HR: 2.20 (1.14, 2.24) 22 years' all-cause mortality HR: 1.18 (1.02, 1.38)	CRAE: 1st vs. 4th quartile	n.s.

	Study	Study population and study design	Sample size/ response rate	Age range/ Yrs of follow-up	Incidence of morbidity and mortality	Retinal arteriolar characteristics	Retinal venular characteristics
11 2006	Wong TY CHS	Americans Population-based, longitudinal study	1992 115 incident CHD 113 incident stroke	69-97 years Mean follow up of 5 years	CHD HR: 2.0 (1.1, 3.7) CHD HR: 3.0 (1.6, 5.7)	CRAE: 1st vs. 4th quartile	CRVE: 4th vs. 1st quartile
12 2002	Wong TY ARIC	White and African Americans Population-based cohort study	84 women incident CHD 187 men incident CHD	51-72 years Mean follow up of 3.5 years	CHD In Women: RR: 1.3.7 (1.08, 1.72) Acute myocardial infarction RR: 1.50 (1.10, 2.04)	AVR: each SD ↓	
13 2001	Wong TY BDES	Americans Population-based, nested, case-control , longitudinal study	416 CHD or stroke death	43-84 years Mean follow up of 10 years	CHD mortality: OR: 1.9 (1.2, 2.9)	Generalized arteriolar narrowing:	n.s.
14 2007	Wang JJ BDES and BMES	Pooled data analysis	7497 out of 8550 (88%) 653 CHD death 299 Stroke death	43-97years Mean follow up more than 10-12 years	Stroke-mortality: HR: 1.64 (1.00, 2.67)	CRAE: 1st vs. 5th quintile	n.s.

	Study	Study population and study design	Sample size/ response rate	Age range/ Yrs of follow-up	Incidence of morbidity and mortality	Retinal arteriolar characteristics	Retinal venular characteristics
15 2007	Klein R WESDR	American whites Population-based, cross-sectional study	1370 with type 2 DM at baseline	30+ years 10 years, 14 years and 22 years' follow-up	22 years' Stroke mortality: HR: 1.71 (1.20, 2.44) 22 years' stroke mortality HR: 1.47 (1.04, 2.07)	 CRAE: 1st vs. 4th quartile	CRVE: 4th vs. 1st quartile
16 2006	Wong TY CHS	Americans Population-based, longitudinal study	1992 115 incident CHD 113 incident stroke	69-97 years Mean follow up of 5 years	Stroke HR: 2.2 (1.1, 4.3)		CRVE: 4th vs. 1st quartile
17 2010	Wieberdink RG Rotterdam study 1990-1993	European Caucasians Population-based prospective longitudinal study	5518 out of 7983 78% 623 incident first-ever stroke: 50 hemorrhagic, 361 ischemic, 212 unspecified	≥55 years Mean follow-up 11.5 years	All stroke: HR: 1.20 (1.09, 1.33) Intracerebral hemorrhages: HR: 1.53 (1.09, 2.15) Cerebral infarctions: HR: 1.29 (1.13, 1.46)	CRAE: n.s.	CRVE: each SD ↑ (SD=20.8 µm)

	Study	Study population and study design	Sample size/ response rate	Age range/ Yrs of follow-up	Incidence of morbidity and mortality	Retinal arteriolar characteristics	Retinal venular characteristics
18 2011	De Jong FJ Rotterdam study 1990-1993	European Caucasians Population-based prospective longitudinal study	5553 out of 6432 86.3% Vascular Dementia	≥55 years Mean follow-up 11.6 years	Vascular dementia: HR: 1.44 (1.10, 1.89)	n.s.	CRVE: Each SD ↑ (SD=20.8 μm)
19 2005	Ikram MK Rotterdam study	European Caucasians with normal glucose tolerance test Population-based prospective longitudinal study	490 without dementia	60-90 years Follow up 3-5 years	Periventricular WML progression OR: 1.74 (1.02, 2.95) Subcortical WML progression OR: 2.50 (1.30, 4.81)	n.s. n.s.	CRVE: each SD ↑
20 2002	Kwa VI	Prospective cohort	179 patients 108 developed SVD		White matter lesions (WML) and lacunar infarcts: Risk ↑	CRAE: ↓	n.s.

Abbreviation: LSOCA: Longitudinal Study of the Ocular Complications of AIDS; MESA: Multi-Ethnic Study of Atherosclerosis; WESDR: Wisconsin Epidemiologic Study of Diabetic Retinopathy; EDC: Pittsburgh Epidemiology Diabetes Complications study; BDES: the Beaver Dam Eye Study; BMES: the Blue Mountains Eye Study; CHS: the Cardiovascular Health Study; ARIC: the Atherosclerosis Risk in Community.

Table 25. Associations between Retinal Vascular Parameters and Incidence of Eye Diseases

	Study	Study population and study design	Sample size and response rate	Age range/ yrs of follow-up	Incident of eye diseases	Retinal arteriolar caliber	Retinal venular caliber
1 2011	Kalyani PS LSOCA	Longitudinal observational cohort study	1250 HIV patients	38-48 years Mean follow-up 6.8 years	Visual function:	n.s.	
2 2008	Tan AG BMES	Australians white Population-based cohort study	3654 baseline 2335 1952 in 10 years' follow-up 82.4%	≥49 years 5-10 years' follow-up	Nuclear cataract OR: 0.62 (0.42,0.92) PSC cataract: OR: 2.40 (1.34,4.29) Cataract surgery: OR: 1.52 (1.06, 2.17)	1^{st} vs. 4^{th} quartile	n.s.
3 2006	Liew G BMES	Population-based, prospective cohort study	106 eyes developed age-related maculopathy (ARM)	10 years' follow up	Early-stage ARM lesion or late-stage ARM:	n.s.	
4 2005	Ikram MK Rotterdam study	Population-based prospective longitudinal study	4345 Caucasians Age-related macular degeneration and open-angle glaucoma	≥55 years Mean follow-up 5.2 years	AMD and OAG: .	n.s.	

Abbreviation: LSOCA: Longitudinal Study of the Ocular Complications of AIDS; BMES: the Blue Mountains Eye Study.

1.5 Pathophysiology of Retinal Microvascular Changes

The evaluation of retinal microvascular characteristics through direct visualization with the ophthalmoscope has been demonstrated to be subjective and unreliable.[188, 189] Retinal photography and grading system have been greatly improved with technical developments. After adopting these techniques for several pioneer population-based studies like BMES, ARIC, CHS, SiMES, WESDR, BDES, the Rotterdam Study in adults and SCORM and SCES in children, retinal microvascular assessments have been proven to be precise and reliable with the non-invasive advantage.[48, 62, 83, 111, 117, 155, 162, 190, 191] However, the underlying mechanisms of retinal vascular parameters changes including retinal arteriolar narrowing, retinal venular widening, larger retinal vascular tortuosity, and larger retinal vascular fractal dimension are still lacking.

It has been postulated that retinal vascular caliber changes might reflect the cumulative structural vascular damage from multiple processes.[29, 39] As introduced in the early chapters, these processes include aging, long-term hypertension, diabetes, arteriosclerosis, inflammation and endothelial dysfunction. Variations in arteriolar and venular caliber may also be influenced by physiological blood flow parameters such as oxygenation and shear stress.[192, 193]

1.5.1 Mechanisms of Retinal Arteriolar Caliber Changes

The pathophisiological changes in retinal arterioles in response to blood pressure elevation are well documented and generalized retinal arteriolar narrowing is one of the earliest signs of hypertensive retinopathy.[3] Firstly, narrowing of arteriolar caliber is part of the initial stages of hypertensive retinopathy. Elevated blood pressure initiates vasospasm and an increase in

vasomotor tone due to local autoregulation, leading to consequent increment in capillary pressures and flows.[1, 3, 193] When elevation in blood pressure continues, chronic arteriosclerotic changes such as intimal thickening, media-wall hyperplasia and hyaline degeneration might develop.[1, 3, 193] Secondly, all these changes manifest an exudative stage which follows with the breakdown of the blood-retinal barrier as a result of autoregulation failure caused by severe elevation in blood pressure.[1] Impairment of autoregulation in the retinal circulation has been implicated in the pathogenesis of various retinal diseases, including diabetic retinopathy, diabetic maculopathy and glaucoma.[194-196] Thirdly, nitric oxide (NO)-dependent endothelial dysfunction has been hypothesized as a key feature of underlying mechanism involved in retinal arteriolar narrowing. Recent studies have shown that NO synthase may have a vasoregulatory role in the retina[193, 197] and linkage regions of retinal vessel caliber overlapped with those of hypertension,[17, 156] both of which provided a strong genetic pathophysiological basis for the speculation of endothelial dysfunction on retinal arteriolar caliber changes.

1.5.2 Mechanisms of Retinal Venular Caliber Changes

There is less understanding of the pathophysiological mechanisms of retinal venular caliber changes. Epidemiological studies reported associations between retinal venular caliber changes and obesity or diabetes.[6, 160] Animal studies demonstrated that administration of lipid hydroperoxide into the vitreous humour of rats increased the number of leukocytes in the retinal microvasculature and also the size of retinal venule caliber, but not arterioles.[198] Furthermore, a series of inflammatory biomarkers such as white blood cell count, IL-6, C-reactive protein and serum Amyloid A.[9, 27, 28, 82-85] have been suggested to be associated with

retinal venular widening in adult population. Since metabolic syndromes like obesity and diabetes were linked with increased blood volume and leptin levels,[199, 200] which might modulate vascular caliber through local mechanisms involving NO release,[201] inflammation,[65] oxidative stress[55, 83] and hyperleptinemia,[106] the association of inflammation with endothelial dysfunction and further with retinal venular caliber changes were postulated.

Furthermore, oxygen saturation has been reported to be associated with retinal venular widening. De Jong et al. investigated 696 European Caucasians with their arterial oxygen saturation (SO_2) and retinal vascular caliber and found that people who had a lower SO_2 (<96%) had a 5.6 µ wider retinal venular caliber ($p<0.01$) than those with a higher SO_2 (≥96%).[55] Therefore, oxygen saturation might partly regulate the polymorphism of retinal venular caliber.

1.5.3 Mechanisms of Retinal Vascular Tortuosity, Branching Angle and Fractal Dimension Changes

The vascular network is believed to be organized to minimize shear stresses and work across the system, and these parameters may provide an indication of how closely a given network conforms to the geometrical ideal.[5] In contrast to retinal vessel caliber measurements, which are generally performed at a fixed location relative to the optic disc and which measure only a single property of the retinal vessel, geometric network measures provide a better indication of the overall health of the vascular system.[5] Taken in totality, these observations of retinal vascular caliber, tortuosity, branching angles and branching coefficient, showing that vascular architecture develops in a way that is optimized for efficient flow, and that deviations from this optimal state occur in disease processes. Thus,

a “global” measure summarizing the whole branching pattern as fractal dimension of the retinal vascular tree as a single parameter would clearly be useful. A fractal is a type of geometric pattern that permits the characterization of objects that branch repeatedly, such as the blood vessels in the heart and lungs. It can be summarized by the fractal dimension, which measures the complexity and density of the branching pattern of retinal vessels and is usually a ratio without unit.[40, 41, 141, 202]

Even though the pathophysiological mechanisms are unclear, the mechanisms suggested in retinal arteriolar and venular caliber changes were also applied to the changes in retinal vessel tortuosity, branching angle and fractal dimension.[5] For example, vessel widening and elongation are known to be caused by high flow rates and vascular congestion, the amount of sheer stress generated by a given flow rate and vessel curvature would allow one to link degrees of tortuosity with degrees of force acting on the epithelium, which in turn can be associated with cellular responses involving gene transcription and release of mediators.[127, 203, 204] Such evidence supported the tortuosity models incorporated retinal vessel skeleton curvature and specially the vessel caliber. As for mechanisms for bifurcation angles and fractal dimension, remodeling of retinal small arterioles has been postulated in several studies in patients with hypertension and diabetes.[126, 205, 206]

1.6 The Gap in Current Retinal Epidemiological Study

1.6.1 Hypertension in Children and Pregnant Women

Hypertension represents a major public health challenge and is a leading risk factor for the morbidity and mortality of cardiovascular disease and cerebrovascular disease.[3, 207]

In the past decades, research has clearly shown that childhood blood pressure are associated with left ventricular hypertrophy,[208] other cardiovascular risk factors,[209] future risk of developing hypertension,[210, 211] and incident large vessel disease in adulthood such as carotid artery intima-media thickness.[212] However, it remains unclear whether elevated blood pressure in childhood could affect the microcirculation,[62] and how such persistent influences may cause all these related vascular diseases. In part, this is because the microcirculation is difficult to measure in children.

Similarly, chronic hypertension and hypertensive disorders during pregnancy, including gestational hypertension, preeclampsia and eclampsia that occur in approximately 3% and 5-6% of all pregnant women, respectively,[213, 214] are the leading causes of adverse maternal and birth outcomes,[215-217] and they are also associated with increased long-term risks of cardiovascular disease (CVD) morbidity and mortality.[218] Abnormally elevated maternal blood pressure in the second or third trimester is associated with poor maternal and neonatal outcomes, including preeclampsia, preterm delivery and low birth weight.[219-221] However, it remains unclear whether elevated blood pressure in childhood and pregnancy could affect the microcirculation, and how such persistent influences may cause related vascular diseases and even on the next generation. In part, this is because the microcirculation is difficult to measure in young children and pregnant women.

1.6.2 Obesity in Children and Pregnant Women

A body of evidence has shown that childhood overweight status and obesity can adversely affect almost every organ system and cause relevant disorders such as asthma, pubertal advancement, glomerulopathy and lower-limb malalignment, and it is associated with increased risk of many chronic diseases in adulthood like

type 2 diabetes, hypertension, cardiovascular disease and gastro-oesophageal reflux.[76, 222-226] Strong evidence from autopsy and epidemiological studies has indicated such relationship between CVD risk factors caused by obesity early in life and vascular signs of atherosclerosis later in life. Two postmortem studies in children and youth autopsy—the Bogalusa Heart Study and the Pathobiological Determinants of Atherosclerosis in Young (PDAY)—reported association between early atherosclerotic lesions in the aorta and coronary arteries and a range of CVD risk factors such as LDL-C and triglycerides [227, 228]. Three longitudinal studies on young and middle-aged adults—The Bogalusa Heart Study, The Cardiovascular Risk in Young Finns Study and The Muscatine Study—also reported similar results that current higher carotid intima-media thickness (IMT) could be traced back to higher levels of CVD risk factors in childhood and adolescenthood [212, 229, 230].

Also, substantial evidence showed that maternal obesity is a risk factor for gestational complications such as hypertension, diabetes and pre-eclampsia, and those postpartum rates of hyperlipidemia and cardiovascular diseases are increased.[231-234] It has been hypothesized that small vessel endothelial dysfunction may partly underlie the link between maternal obesity and adverse outcomes with a microvascular component both in children and pregnant women,[235] but the contribution and exact role of the microcirculation during childhood and pregnancy is unclear due to difficulties in its assessment.

1.6.3 Pathology of Myopia in Children

According to high socioeconomic cost in high prevalence of myopia in Asian Chinese (82.2% in Singapore young population[236]) and high prevalence of depression in Asian pregnant women (12.2% in Singapore pregnant population[237]),

both of which had adverse pathologic influence on retinal circulation through different pathways, the study on myopia and antenatal mental health with retinal circulation might help to better understand pathophisiological changes on microcirculation in vivo caused by structural condition like myopia and mental condition like depression, anxiety and poor sleep quality.

1.6.4 Antenatal Negative Emotion in Pregnant Women

Depression, anxiety and poor sleep have been well established to be associated with cardiovascular diseases both in general population and clinical patients with obesity or coronary heart disease.[238-242] Growing evidence also shows that depression and anxiety during pregnancy is related to adverse maternal outcome such as preeclampsia[243] by possibly sharing similar pathophysiology with cardiovascular disease (CVD).[244] Since antenatal depression is commonly accompanied by anxiety and poor sleep[245, 246] and it affects up to 12.8% of pregnant women[247] in Caucasian population and 12.2% in Singapore population[237] during the second trimester, antenatal mental health has become an important public health issue for its influence on further vascular complications. Therefore, it is necessary to study the precise pathophysiological mechanisms to enlighten the association of antenatal depression, anxiety and poor sleep with hemodynamic circulation.

1.6.5 The Major Gap in Retinal Epidemiological Studies

This literature review has summarized findings: 1) from the past 10 years' population-based epidemiological studies (from 2000 onwards) regarding determinants of retinal vascular parameters in terms of systemic factors, environmental factors, genetic factors, diet and medicine intake, and medical condition factors (e.g. physical activity, hypertension, obesity and diabetes); 2)

from the past 10 years' population-based epidemiological studies (from 2000 onwards) regarding predictive values of retinal vascular parameters in incidence of major optical condition (e.g. glaucoma, myopia and cataract), cardiovascular diseases (e.g. ischemic heart disease and left ventricular hyperplasia), cerebral disease (e.g. lacunar stroke) and renal diseases (e.g. chronic kidney dysfunction).

Associations between retinal vascular parameters changes and all types of risk factors in general populations have provided potential clinical importance, such as reflecting different pathophysiological processes in major clinical outcomes (hypertension, diabetes, stroke, cardiovascular diseases, etc.) and increasing predictive value of incorporating retinal vascular caliber measurements into traditional cardiovascular prediction models (Framingham risk models). However, these associations have not been established in very young children and pregnant women, two specific populations with possible early predisposition of major systemic diseases.

In summary, a range of systemic and environmental risk factors have profound effects on the variation of the retinal vascular parameters in general population. However, this impact is not established in special population such as pre-schoolers and pregnant women. Also, the predictive values of retinal vascular parameters changes in shorter occurrence of gestational hypertension, gestational diabetes and baby growth have not been explored. Determining these specific influences may allow greater understanding and possible prevention of complex polygenic human diseases from very early life or even during pregnancy. In order to fill up the gap of such retinal studies, our study aimed to determine the risk factors for a series of retinal vascular parameters changes both in young children and pregnant women in Singapore.

CHAPTER TWO

2. Methods

2.1 Objective

2.1.1. General Objective

To study the major risk factors for changes in retinal vascular parameters among Singapore Chinese children and Singapore pregnant women.

2.1.2. Specific Objective

1. To study the relationship between blood pressure and retinal vascular parameters among children and pregnant women.
2. To study the relationship between body fatness indices and retinal vascular parameters among children and pregnant women.
3. To study the relationship between systemic diseases such as hypertension and diabetes and retinal vascular parameters among children and pregnant women.
4. To study the relationship between refractive error and ocular biometric parameters and retinal vascular parameters among children.
5. To study the relationship between antenatal mental health and retinal vascular parameters among pregnant women.
6. To study the predictive value of retinal vascular parameters on gestational complications including gestational hypertension, pre-eclampsia and gestational diabetes among pregnant women.

2.1.3 Hypothesis for Risk Factors

From the literatures, we hypothesized that a series of risk factors such as blood pressure, body mass index, subcutaneous skinfold thickness, refractive error, negative emotion such as depression and anxiety and sleep quality are potentially associated with retinal vascular parameters changes among children and pregnant women. Retinal vascular parameters potentially carry predictive values in incident gestational hypertension, gestational diabetes and pre-eclampsia in pregnant women.

If the associations are established by our study, known risk factors for late life CVD and systemic diseases are also associated with micropathology in young children and pregnant women. Since it has been suggested that late life CVD risk factors can be predisposed early in life and in utero, our findings provided etiological evidence on this theory hypothesized by Barker and colleagues. Furthermore, it will be necessary to follow up both populations and even children given birth by our pregnant subjects, in order to investigate whether retinal vascular parameters over time is predictive of long-term systemic diseases and even CVD developments. Until these associations are established we are not able to carry current message on retinal imaging to public health practice or patient care, such as early detection for disease outcome and indicative for clinical intervention or treatment.

A. Scheme One--STARS and STARS Family Retinal Study

2.2 Study Designs

This is a cross-sectional study which is part of "A Study on Strabismus, Amblyopia and Refractive Error Study in Singaporean Chinese Preschoolers (STARS)" and STARS Family study.

STARS is a population-based, cross-sectional study conducted from May 2006 through October 2008. Chinese children 6 to 72 months of age and living in government Housing Development Board (HDB) apartments (85% of the population) in the Western regions of Singapore (Jurong East and Jurong West) and Southwestern regions of Singapore (Bukit Batok, Clementi and Queenstown) were invited to participate. These areas were chosen due to their location close to the Singapore National Eye Centre (SNEC) and Jurong Medical Centre where the subjects are interviewed and examined. The household address database was obtained from the Ministry of Home Affairs. Clinic invitations were sent out through mails and were followed by phone calls and a home visit. Children who were non-Chinese, underage or overage, chronic medical or mental conditions, were not living in the household address for the previous 6 months, or who had moved from the resident address were excluded. Among 4164 eligible Chinese children, 3009 participated in the study (72.3%).

STARS Family study is a cross-sectional study conducted from March, 2008 to March, 2010. It is a family-based genetics study of early-onset myopia in

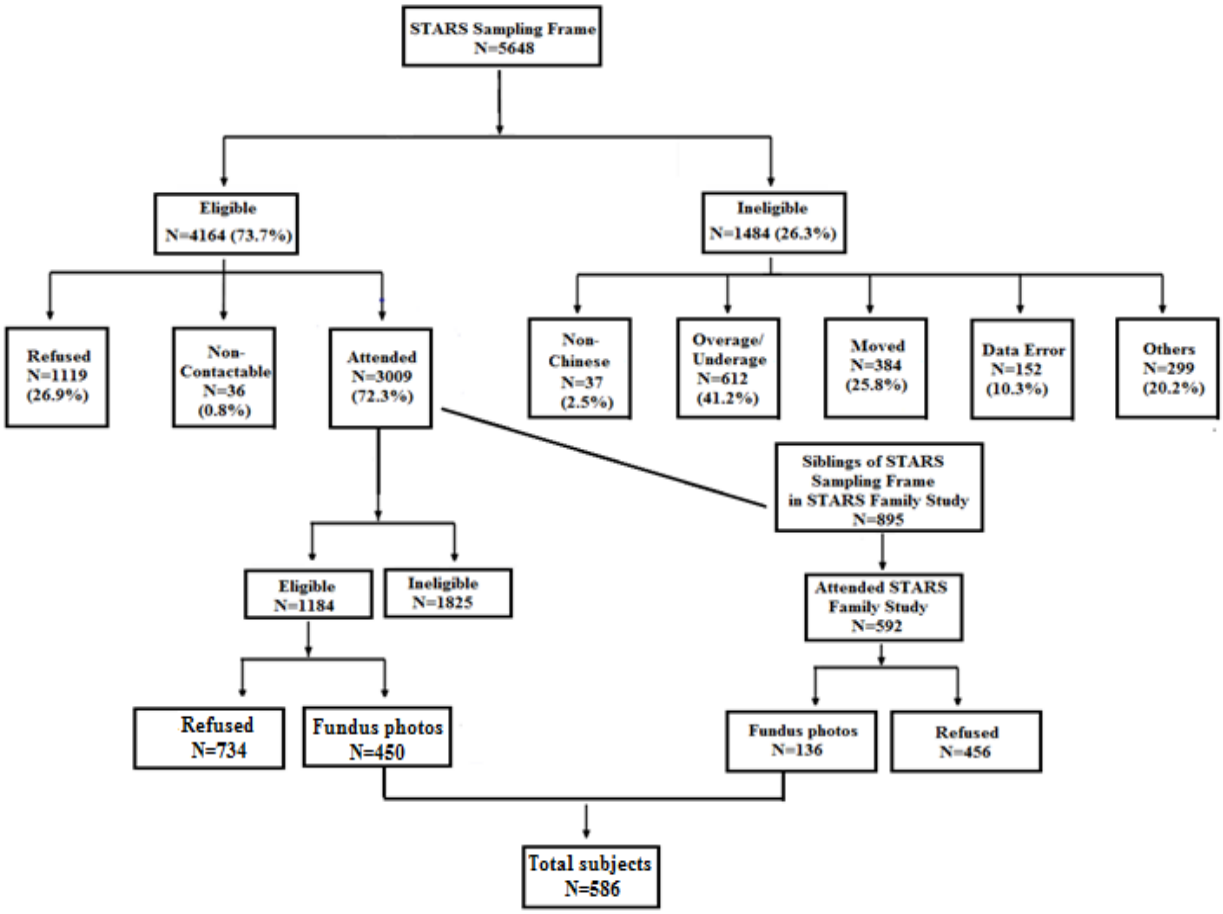
Singapore children. Siblings of children who have already participated in STARS study were invited this STARS Family study. 895 subjects were screened while only a total of 592 children aged 4-16 years were recruited (66.1%).

Both STARS and STARS Family study followed the tenets of the declaration of Helsinki. Informed written consent was obtained from parents after explanation of the nature and possible consequences of this study. It was approved by Institutional Review Boards (IRB) of the Singapore Eye Research Institute (SERI) and the National Healthcare Group (NHG).

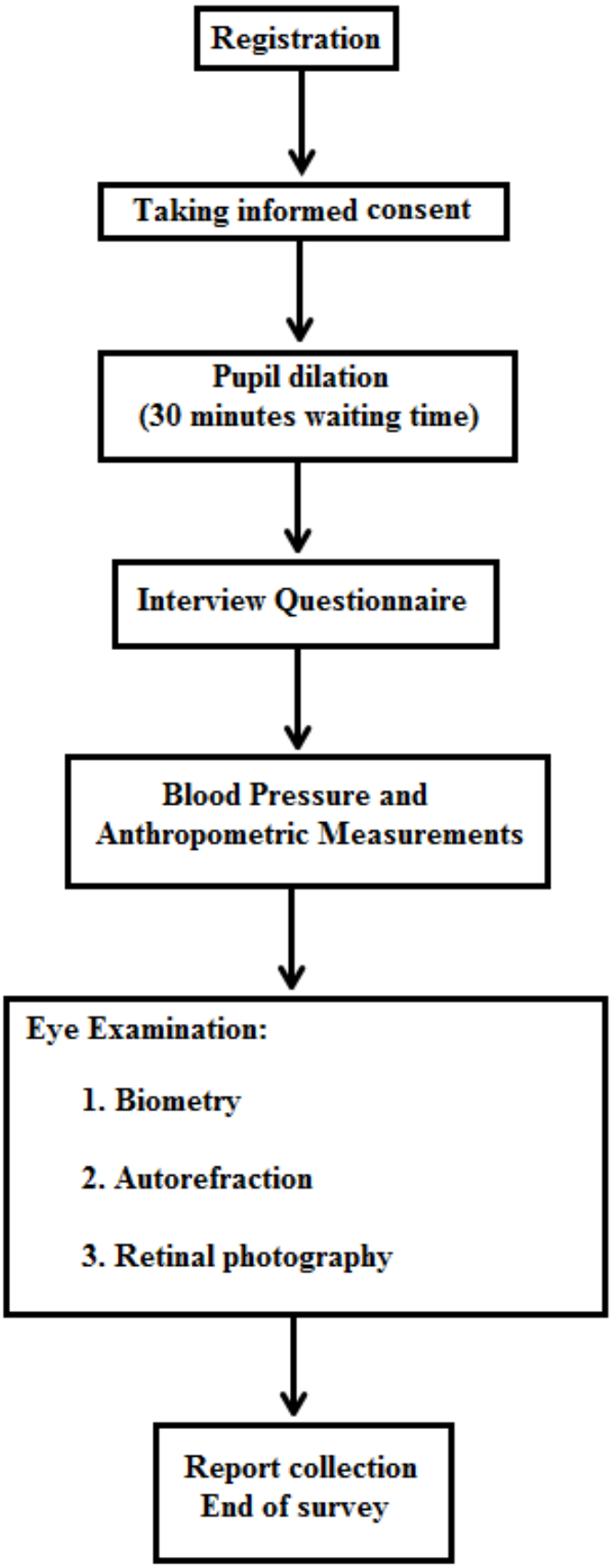
2.3 Inclusion and Exclusion Criteria

Children who were at least 4 years old and participated either in STARS or STARS Family study would be eligible for our retinal study. Children who were not Chinese, not willing to take eye drops for pupil dilation, with Nystagmus or other media opacity, not subjects recruited from either of STARS or STARS Family study were excluded. In total, 586 children aged 4 years and above were recruited in our retinal study.

2.4 Recruitment Flow


STARS Sampling Frame
N=5648
Eligible
N=4164 (73.7%)
Ineligible
N=1484 (26.3%)
Refused
N=1119
(26.9%)
Non-
Contactable
N=36
(0.8%)
Attended
N=3009
(72.3%)
Non-
Chinese
N=37
(2.5%)
Overage/
Underage
N=612
(41.2%)
Moved
N=384
(25.8%)
Data Error
N=152
(10.3%)
Others
N=299
(20.2%)
Siblings of STARS
Sampling Frame
in STARS Family Study
N=895
Eligible
N=1184
Ineligible
N=1825
Attended STARS
Family Study
N=592
Refused
N=734
Fundus photos
N=450
Fundus photos
N=136
Refused
N=456
Total subjects
N=586

2.5 Clinic Visit



2.6 Clinic Visit and Ethnics Consideration

After detailed explanation of the study purpose by the trained staff, parents would sign the consent form on behalf of their children. Two copies were signed. One was given to the parents and the other was kept as study record. If the parents or children refused to participate part of the examinations or the whole research process due to any possible reason, their decision would be respected and certain examination would be skipped or even the whole survey would be discontinued.

After registration, risks and benefits of our retinal study were explained by qualified optometrist. Also, parents or guardians were informed of the confidentiality and autonomy with their children throughout the whole study.

2.7 Study approval

The STARS and STARS Family retinal study was funded by the National Medical Research Council (NMRC/1009/2005, NMRC/1112/2007 and NMRC/1176/2008), and approved by Institutional Review Boards of the Singapore Eye Research Institute (SERI) and the Singapore National Healthcare Group (NHG). The study was conducted according to the tenets of the declaration of Helsinki. Informed written consent was obtained from parents prior to any testing.

2.8 Clinical Examinations

2.8.1 Blood Pressure Measurements

Blood pressure was performed on children aged 48 months old and above. It will also be measured using the automatic Omron sphygmomanoter (Omron HEM 705 LP, Omron Healthcare Inc, USA) using the appropriate pediatric cuff size, after a period of rest. The cuff size was selected to ensure that the bladder spans the circumference of the arm and covers at least 75% of the upper arm without obscuring the antecubital fossa. The average of 3 readings was taken.

2.8.2 Anthropometric Measurements

Height was measured standing and without shoes or socks according to a standard protocol. Height was recorded to the nearest 1.0 mm.

Weight was measured using the SECA (Vogel and Halke, Germany) weighing sale. If the child was not willing or unable to stand on the scale assisted, the guardian carried the child and the combined weight was assessed. Subsequently, the weight of the guardian was measured. The difference of the weights was the child's weight. Weight was recorded to the nearest 0.1 kg.

Triceps skinfold measurement was performed on children aged 48 months old and above, and was made in the right side of the body. Three vertical skin fold measurements were taken half the distance between the acromion process (prominent bone at top of shoulder) and the olecranon process (elbow bone). The caliper (Holtain Ltd, Crymych, Wales, UK) was placed 1 to 2 cm away from the

thumb and finger, perpendicular to the skin fold, halfway between the crest and the base of the fold. Triceps skinfold was measured to the nearest 0.1 mm.

2.8.3 Eye Examination

Pupil dilation with eye drops Cycloplegic objective refraction was assessed approximately 30 minutes after topical instillation of 3 drops of 1% cyclopentolate and 2.5% phenylephrine, given 5 minutes apart. Further interview while dilating cycloplegic eye drops were administered to paralyze excessive accommodation and to prevent pseudomyopia.

Ocular Biometrics Axial length, corneal curvature and anterior chamber depth measurements were obtained monocularly using the noncontact partial coherence interferometer Zeiss IOLMaster in children. A total of 5 consecutive readings were obtained for axial length, corneal curvature and anterior chamber depth, with a signal-to-noise ratio of more than 2.0. The mean of the 5 readings was used in the analysis. IOLMaster testability was defined as the measurement of at least 2 axial length readings.

Table-Mounted Autorefraction. Cycloplegic autorefraction and keratometry were performed using the Canon Autorefractor RK-F1 (Canon, Tokyo, Japan) in children, where a total of 5 consecutive readings are obtained. The autorefractor readings were acceptable if the difference between the lowest and highest reading was 0.25 diopters or less. Testability for autorefraction was defined as at least 2 readings for sphere and cylinder.

2.9 Fundus Photography Examination

2.9.1 Training Program

This training program was arranged as a 3-week course. The first week was to introduce equipment, photography technique and hands-on operation of equipment. The second week was to learn how to export captured images, save data, back-up data, and continue hands-on operation of equipment. The third week was to test for competency and validation.

2.9.2 Training Assessment

Out of 200 retinal photos taken from healthy subjects (50, 25%) and elderly patients (150, 75%) with glaucoma or cataract, 185 (92.5%) retinal images were gradable with good quality. The other 15 (7.5%) retinal photos were ungradable due to the patients' late stage of cataract.

2.9.3 Retinal Photography Examination

Subject Explanation. It was important to reassure the patient that there was no damage caused by the flashes of light from the camera. The patient was expecting a flash with each photos taken. The photos included the macula (area of central vision) and it was normal to see a blue or red tint immediately after the flash is released. However, it would disappear within two to three minutes. It was important to emphasize to the patient that there will be no direct contact to the eyes in anyway. A sample script of a typical retinal photography explanation

(suitable for use as written material for deaf or anxious patients) was elaborated as follows:

We will be taking several photographs of the back of both of your eyes (the retina) so as to study the blood vessels and to look for any abnormalities. We will not be touching your eyes. We require you to sit in a darkened room before a special camera with your chin resting on the chin-rest. We will align and focus the camera on your retina. During the aligning process you will see some small red lights, a blinking green box and red bars. We will ask you to follow the blinking green whichever position we move it to. Just before we take the photo, you will be asked to blink your eyes few times and then open them as wide as you can. The camera will release a bright light (flash) with each photo taken Immediately you will see a blue or red circular spot with the photographed eyes. This will disappear within 2-3 minutes. Please note that we are only taking pictures and it is not an x-ray. You will be notified should we find anything which requires urgent attention.

Retinal Photography. First step was to switch on the camera and the desk-top computers (There are two "on" buttons for the camera and one "on" button for the computer.), then we cleaned the chin-rest and the head-rest with alcohol swipes. Besides, we needed to ensure that the subject's chin was resting comfortably on the chin rest and forehead touching the head-rest. After keying in Patient ID twice, Patient's Name and Patient's Gender, it was required to adjust the machine so that

the eye-level was aligned with the red marking on the machine. After this step, we pressed the square button which was on the machine to go into the fundal view, again to adjust the alignment bar on the screen. If it was not aligned, we would align it by turning the knob which can be found at the side of the machine.

We instructed the patient to follow a green colored dot (fixation light) which he/she could see through the camera lens by moving the round button situated near to the joystick. Then we moved the round button until the subject's optic disc was in the center. There were two white dots (notches) on the screen as guidance. We had to make sure that the two notches are at the center of the screen by turning the joystick up and down, inward and outward until they were clear. As soon as the two white dots were clear and at the center of the screen, we pressed the button of the joystick to take the photo. When the photo was downloaded completely, we proceeded to take the macular photo by moving the round button (fixation light) until the subject's optic disc was at the side.

Once done in taking fundus photos, we clicked "end" on the monitor and through clicking on "Select All" and "Export Selected" to save images into the "the appropriate folder" (targeted folder). However, if the quality of images was poor, like with artifacts on, with dark patches due to undilated pupils or white in color because the subject had blinked, it was necessary to select those poorly taken photos and click on "DELETE" button for deletion. If the current day's folder had not been created, we clicked on "Make a New Folder" and created the

current day's folder in this format "YYYY-MM-DD". Once creating a new folder, we saved the image into the sub-folder named after the Patient's Study ID.

Fundus photos were captured on 3-standard fields of a child's right eye. The first photograph was centered on the optic field **Field 1** (**Figure 4**). The second photograph was centered on the macula field **Field 2** (**Figure 4**). The third photo was with the optic disc slightly off-centre temporally **Field 3** (**Figure 4**). The following are descriptions of the standard fields.

Field 1 - Disc: Centre the optic disc at the intersection of the cross hairs in the ocular. **Field 2** - Macula: Centre the macula near the intersection of the cross hairs in the ocular. To keep the central gray artifact (which can be created by some cameras) from obscuring the centre of the macula, the intersection of the cross hairs should be placed about 1/8-1/4 DD above the centre of the macula. A suitable position could often be obtained by rotating the camera temporally from the Field 1 position, without vertical adjustment. **Field 3** - Modified Field 1 - Place the optic disc slightly off centre temporally to the intersection of the cross hairs in the ocular.

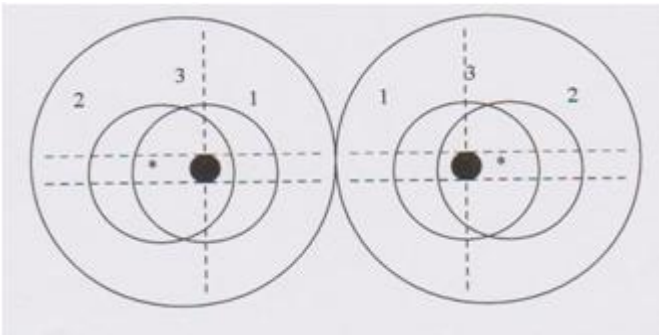


Figure 4. **3-Standard Fields of Fundus Photos of Both Eyes**

Color photographs of each eye were reviewed and assigned a grade for overall quality. The grades included three indicating that a set can be evaluated with no problem (***excellent, good, fair***), two signifying that a set can be assessed although quality interferes somewhat (borderline-explained, borderline-unexplained), and three indicating that a set cannot be completely evaluated (inadequate-explained; inadequate-unexplained).

2.10 Interview at the Clinic

Interviews with a parent of guardian will continue to be conducted during the clinic visit. The questionnaire was translated into Chinese. The questionnaire was piloted in approximately 100 parents of similarly aged children and revisions to the questionnaire were made after discussion with the parents. The interviews are conducted by a study research assistant and take approximately 30 minutes to complete.

2.10.1 Demographic and Socioeconomic Status

Demographic risk factors such as age, gender, total family income, father's and mother's completed educational level were collected. Parents were asked to give information on their total combined monthly household income with the range: "less than S$1,000", "S$1.000 – S$2,999", "S$3,000 – S$4,999" or "more than S$5,000". Example of questions on father's education are: "What's the father's completed educational level?" with the responses: "None", "Primary", "Secondary", "'O' / 'N' Levels", "'A' Levels/ polytechnic/ Diploma/ ITE/

Certificate", "University education (degree and above including bachelor, master and PhD)" or "Others". The questions on mother's education were similar.

The age when the biological mother gave birth to the child was collected. Maternal antenatal condition on anemia or low blood count, high blood pressure, diabetes and other problem during the pregnancy were reported during the clinic interview.

2.10.2 Parental Life Style

Information on smoking history was obtained from the parents. Mothers were asked if she had ever smoked at least one cigarette a day for the one year or longer with the responses: "No", "Yes and she currently smokes" or "Yes and she quit smoking". If the mother had quit smoking, she was asked when she had quit smoking. If there was a history of smoking, the mother was asked to give the age which she first started to smoke cigarettes on a regular basis. Lastly, the number of cigarettes smoked by the mother was ascertained and categorized into: "less than 6 cigarettes", "7 to 12 cigarettes", "13 to 22 cigarettes", "23 to 32 cigarettes", 33 to 42 cigarettes" or "more than 43 cigarettes". Father's smoking history was ascertained with the same questions the mother had answered.

The history of smoking and alcohol drinking during pregnancy was obtained from the biological mother. She was asked "At any time during the pregnancy with the child, did you smoke/drink alcohol?" If the biological mother had ever smoked or drunk during her pregnancy with the child, she was asked to provide

information regarding the months (eg. 1st month, 2nd month, etc.) of the pregnancy, such as the number of cigarettes she smoked per day or the numbers of drinks she had per day.

2.10.3 Birth Data from the Health Booklet

Birth history data were acquired from a documented medical record booklet (Health booklet) – the hospital doctor or nurse records details of the birth history a few days after birth. Birth parameters documented include birth weight (grams), gestational age (weeks), head circumference (cm) and birth length (cm). Parents were requested to bring the child's health booklet to the clinic for the clinic interviewer to record the birth parameters.

2.11 Retinal Vessel Caliber Assessment With IVAN Software

2.11.1 Grading Institute

The retinal vessel diameter measurements were done by retinal vessel diameter grading team in SERI, whose office was located in Level 10, Fushionopolis, North Buona Vista in Singapore.

2.11.2 Computing and Image Requirements

Hardware equipments were as follows: Windows XP Service Pack 2[i], dual core CPU, dual core CPU, dual head video card supporting for dual monitor, and high resolution as 1280x1024 pixels per screen. Software requirements were as follows: IVAN software as 40MB, Microsoft Excel spreadsheet application with support

for ".CSV" (comma-separated-value) format, image editor for measuring pixel size and upscaling image resolution.

The IVAN was operated of a two-monitor workstation with the image files organized by reading lists. The concept of "zone" adopted from the Modified ARIC grid was used in the IVAN; it composed of 3 concentric circles which demarcate an average optic disc, Zone A defined as the region from the disc margin to ½ disc diameter from the disc, and Zone B defined as the region from 1.2 disc diameter to 1 disc diameter from the disc. All retinal vessels wre measured in Zone B. Following the Knudtson's revised formula to calculate the **C**entral **R**etinal **A**rtery **E**quivalent (CRAE) and the **C**entral **R**etinal **V**ein **E**quivalent (CRVE), only the width data from the 6 largest arterioles and 6 largest venules were required. The formula allowed individual vessel diameters to be combined into summary indices reflecting the average arteriolar and venular diameter of that eye, taking into account branching patterns. These summary indice were further expressed as the Artery-to-Vein ratio (AVR), which is simply calculated as CRAE divided by CRVE.

The image parameters set for STARS fundus photos are described as follows: 45 degrees of field size, standard Field 'F1-Disc centered' for retinal vessel diameter measurement, Image Conversion Factor (ICF) to act as scale factor for software as 4.2, minimum resolution of 3000*2000 pixels, and images processed as JPEG format with 90% quality or more.

2.11.3 Procedures for Retinal Vessel Diameter Measurements

First to log onto workstation with study name first and grader's name the second. The application opened on the primary monitor with 3 windows: the image display window, the control window, and the vessel data parameters file. Then drag control window from behind image window to secondary monitor.

Once an image was loaded into IVAN, the software would attempt to mark as many vessels possible, depending on the image quality. Most vessels with a caliber greater than or equal to 50μm could be identified as either arteriole or venule from the digital image. The grader had the options to identify each vessel with Zone B as either an arteriole or a venule and made changes as necessary. At times, the option to view the entire uncropped image might be helpful. To use the Big6 formula, the largest 6 venules and largest 6 arterioles were taken into measurement. If more than 6 were automatically and correctly displayed, no deletion was necessary because the calculating process would ignore the unnecessary values. Through changing or removing colors or through a "finer" view-SPLAT view, the grader could discern the vessel pattern and feasibility of accurate measurements (**Figure 5**).

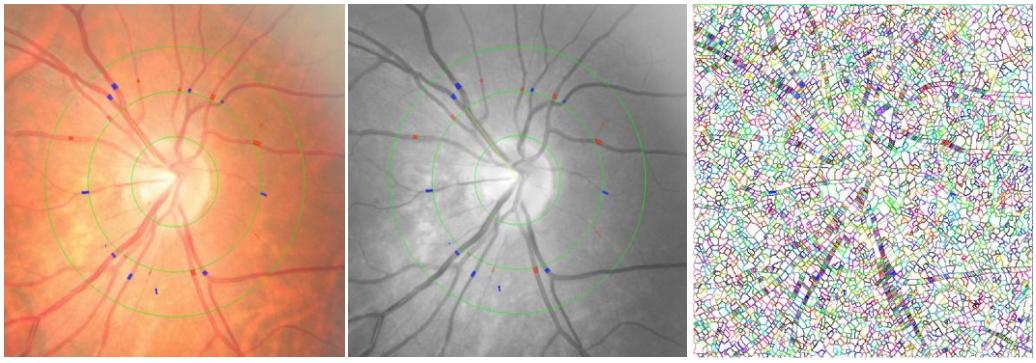


Figure 5A. **Color model** **Figure 5B. Grey Model** **Figure 5C. Splat model**

To make the changes mentioned above to vessels, the vessel must first be assessed. Vessels were assessed by clicking on the seed point (the proximal end of the vessel trace) on the image display or by clicking on the vessel number button on the data table. Both methods highlighted the vessel trace, then we directed the pointer to the vessel trace seed point (and angle location), and highlighted the data for the selected vessel on the data table. In this way, the visual vessel trace and the data (angle, width, Sigma) for each vessel were easily identified (**Figure 6**).

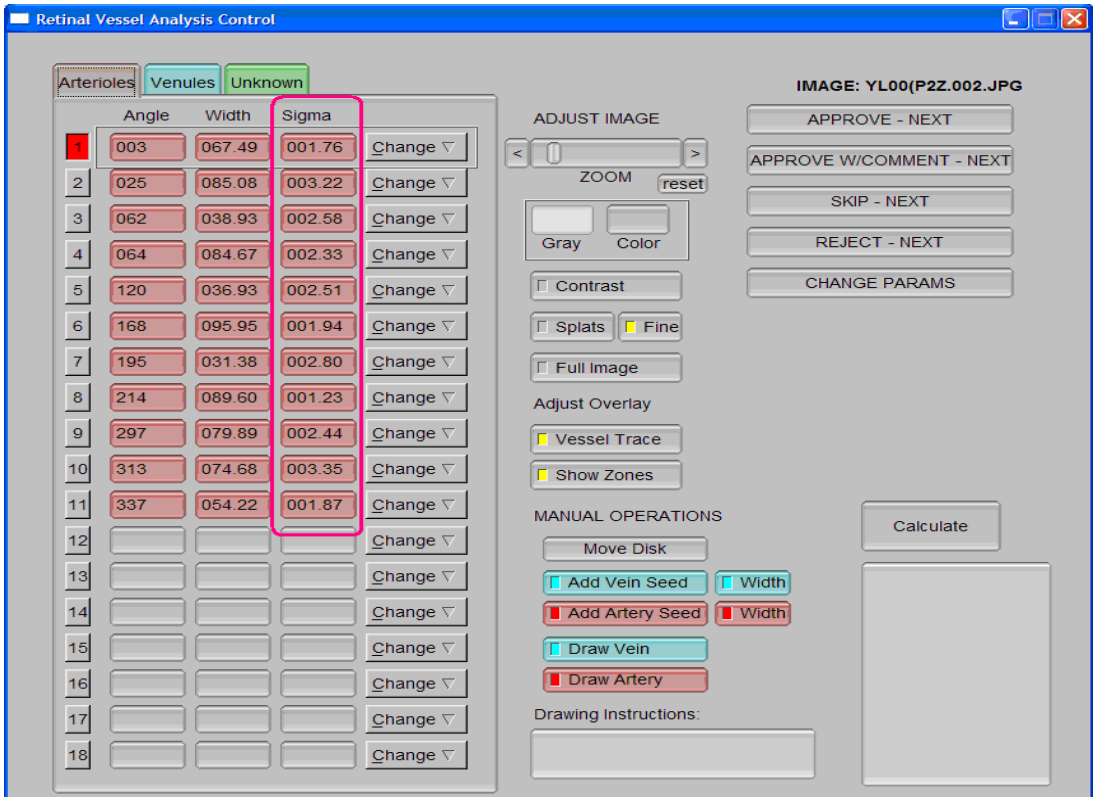


Figure 6A. Retinal Arteriolar Analysis Control

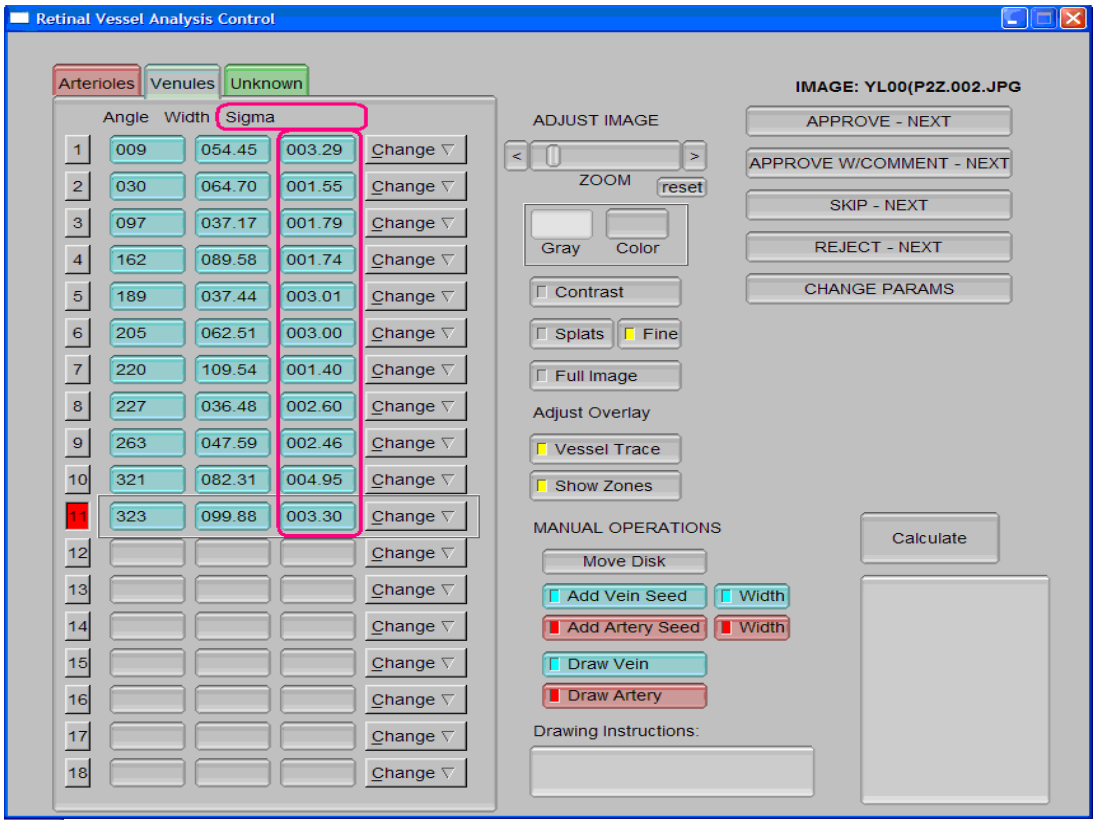


Figure 6B. Retinal Venular Analysis Control

The acceptable measurements were required as follows: the visual vessel trace without obvious outliers from the visible edges of the vessel and the length of the measured segment being as long as possible through zone B for each particular vessel. Graders will use “delete”, “truncate”, “proximal chop” to optimize the automated measurement since it may be difficult to agree on what segment represents a “normal” width. Furthermore, graders could use “Draw Artery” and “Draw Vein” to extend measurement on non-selected vessels. Sigma of each vessel should be modified to less than 8.0. Perceived variations in vessel edges should be allowed, especially if the measured segment is relatively long through Zone B.

At the point of branching when the Zone B gridline fell, the vessel branches would be measured as trunks. All trunks were measured before branching even if the segment measured was acceptable to grade. If suspected branches were impossible to identify with certainty and are of small size, then the grader should not truncate the vessel and instead measures as long a segment as possible.

2.11.4 Data Saving Options

At the completion of grading, there were 5 options for saving the data, which are “**APPROVE-NEXT**”, “**APPROVE W/COMMENT-NEXT**”, “**SKIP-NEXT**”, “**REJECT-NEXT**”, and “**CHANGE PARAMS**”. The “**APPROVE-NEXT**” option was used for images that are gradable and no comment was necessary to move on to the next image. The “APPROVE W/COMMENT-NEXT”

option was used for images that were gradable but might have factors affecting the consistency of the data. These comments were evaluated by the statisticians and listed as follows: Poor Quality, Not Big 6 (artery), Not Big 6 (vein), Not Big 6 (artery and vein), Confounding Pathology, NRE (no right eye), NLE (no left eye). The "SKIP-NEXT" option was for images that did not have to be graded and could be processed to the next image. The "REJECT-NEXT" option was used for images that were ungradable. Ungradable images had no acceptable vessels measured on the initial display. Images with <4 acceptable measurements of either vessel type, or if there was no clear view of the optic disc were considered ungradable.

After choosing the related option and followed by computer guidance, retinal vascular measurements were saved in ".CSV" format automatically. And then they were exported to a study dedicated database stored in central server. In addition, quality assurance testing would be carried throughout the grading period for intra and inter observer reliability.

2.11.5 Confidentiality

All study participants were identified by unique identifiers. All fundus photographs were identified by identification numbers only. Personal information (names, IC numbers, addresses and telephone numbers) was kept in a separate file. Only study staff were given computer passwords to access the study data files. All case forms with identifiers were kept under lock and key.

2.11.6 Data Management

Data were entered onto a pre-designed secure Access database by a data entry clerk. There were built-in range checks and warnings for missing data. A 100% manual check waas performed by another clerk to ensure the accuracy and consistency of the data. A codebook to identify the column variables had been developed. Any change was documented in a data audit trial form. A back-up of the entire database was obtained in a hard drive. In collaboration with Singapore Eye Reaserch Institute and the National University of Singapore, a retinal and ocular image networked storage system (SiRIAN) was tailored and set up for remote access for grading of the collected images. Selection of features based on the different type of images and related diseases were further explored. Image processing techniques to extract these features accurately and robustly from the images were investigated.

2.11.7 Reliability Test of Retinal Vessel Assessments

A single grader, masked to blood pressure measurements and participant characteristics, performed all of the retinal vascular caliber measurements for this study. Intra-grader reliability was assessed in 70 randomly selected retinal photographs, and the intra-class correlation coefficient was 0.98 for CRAE and 0.99 for CRVE.

2.12 Definition of Terms

2.12.1 Childhood Hypertension

The children were categorized as having hypertension by referring to their age-, gender-, and height-specific SBP or diastolic blood pressure in the 95th percentile or more, or both, according to the guidelines provided by the 2004 Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents[248] and the growth charts provided by the American Centers for Disease Control in 2000.[249]

2.12.2 Childhood Overweight and Obesity

The anthropometric measures included height, weight, body mass index and triceps skinfold thickness. Body mass index (BMI) was calculated as weight in kg divided by the squared height in meters (kg/m^2). Overweight and obesity were defined according to BMI and Triceps skinfold thickness, respectively. The top 85^{th}~95^{th} percentile of age-, gender- and height-specific body mass index (BMI) was considered as overweight and the top 95^{th} percentile of age, gender- and height-specific body mass index (BMI) was considered as obesity.[249] The top 85^{th}~95^{th} percentile of age-, gender- and height-specific triceps skinfold thickness was considered as overweight and The top 95^{th} percentile of age, gender- and height-specific triceps skinfold thickness was considered as obesity.[250]

2.12.3 Abnormal Birth Parameters

Pre-maturity was defined as fewer than 37 gestational weeks.[251]

Children with low birth weight were defined as lighter than 2500 g at birth, while children with high birth weight were defined as heavier than 4000 g at birth.[251]

2.12.4 Myopia, Emmetropia and Hyperopia

The right eye data was analyzed because the correlations between refractive data for right and left eye were high. Refractive error was evaluated as a continuous variable and dichotomous variables. Spherical equivalent (SE) was defined as sphere plus half negative cylinder. Myopia was defined as SE at least -0.5 diopters. Emmetropia was defined as SE between -0.5 and +0.5 diopters. Hyperopia defined as SE at least +1.0 diopters.[252, 253]

2.13 Statistical Analysis

The student's t-test and χ^2-test were used to compare means/categorized data of potential variables of interest between group with retinal photography and group without retinal photography according to our outcomes. If the variables of interest were categorized into more than two groups (Mother's Education), the differences among the groups were analyzed by using one-way ANOVA test.

The central retinal arteriolar caliber and central retinal venular caliber were analyzed as continuous variables. Gender, father's education, family income, father's smoking, mother's smoking and smoking and alcohol drinking during pregnancy were analyzed as categorical variables. Anthropometric measurements (blood pressure, weight, height, triceps skinfold), birth parameters (birth weight,

birth length, head circumference, gestation week) and optical biometrical measurements (spherical equivalent, axial length) were categorized into quintile or quartiles and also analyzed as continuous variables. A p value for trend in for consecutive quintiles (systolic, diastolic, and mean arterial blood pressure) and consecutive quartiles (BMI, triceps skinfold thickness, birth weight, gestation week, spherical equivalent, and axial length) was generated.

We used multiple linear regressions to determine the changes in retinal arteriolar and venular calibre per standard deviation (SD) increase in anthropometric variables (SBP, DBP, MABP), birth parameters (birth weight, gestation), and biometric measurements (spherical equivalent, axial length). Models were initially adjusted for age and gender, and then additionally adjusted for multiple variables. A significant p value was defined as <0.05. All statistical analyses were undertaken using PASW 18.0 (SPSS Inc).

B. Scheme Two – GUSTO retinal study

2.2 Study Recruitment

We analyzed data from an ongoing cohort study, the Growing Up in Singapore Towards Healthy Outcomes birth cohort (naturally conceived) and the add-on In-Vitro Fertilization cohort study (conceived through assisted reproductive techniques). Except for differences in conception, both cohorts shared the same inclusion criteria. Pregnant women with 18 years and above, attending the first visit (<13+6 weeks GA) at the maternity units of KKH and NUH, currently

residing in mainland Singapore as citizens or permanent residents, intending to eventually deliver in KKH or NUH and to reside in Singapore for the next 5 years, intending to donate cord, cord blood and placenta and fetus racially homogenous with both sets of grandparents of the same ethnicity were eligible for GUSTO study. Pregnant women on chemotherapy, with significant medical conditions such as Type 1 diabetes mellitus, psychosis, under certain medication such as psychotropic drugs, or mixed marriages were excluded.

The GUSTO birth cohort study's aim was to examine how epigenetic changes detected at birth both reflects past developmental influences and, in association with other factors, influences future trajectories of development and its relationship to metabolic risk. Among the 3335 participants from GUSTO main study and 98 participants from GUSTO IVF study, 1163 and 85 were finally recruited into GUSTO main study and GUSTO IVF study, respectively. Classified by clinic visit, 952 subjects attended KKH study site and 296 subjects attended NUH study site.

2.3 Eligibility

For GUSTO retinal study, only women recruited from KKH were eligible due to logistic restriction. Participants who were not GUSTO main study or GUSTO IVF study subjects, mix-race for the latest three generations, not attending KKH clinic site, unwilling to take retinal photography were ineligible.

The study was approved by both Singhealth Centralized Institutional Review Board and the National Health Group's Domain Specific Review Board. It was

conducted according to the tenets of the Declaration of Helsinki. Informed written consent was obtained from every participant before any testing.

2.4 Study Population

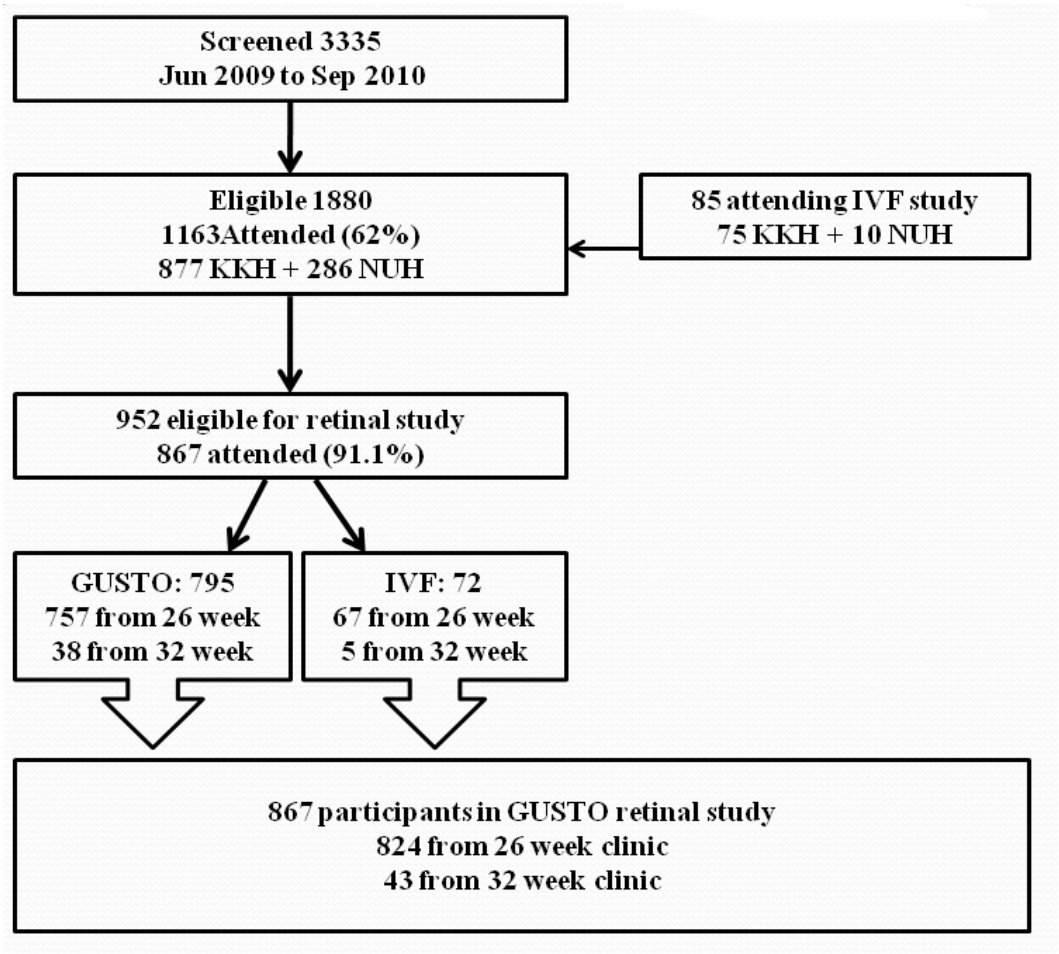


Figure 7. Recruitment Flow of GUSTO Retinal Study

A total of 952 patients were recruited in KKH for GUSTO study. Among these 952 patients, 824 took the retinal examination during 26th week of gestation while 43 had their retinal photographs taken during 32th week of gestation. Within the 824 participants suitable for our retinal study time frame, 757 were recruited from GUSTO main study and 67 were recruited from GUSTO IVF study (**Figure 7**).

2.5 Study Approval

GUSTO is funded by NMRC/BMRC Translational Clinical Research (TCR) (NMRC/TCR/004).

2.6 Clinic Operation Flow

Retinal photography and maternal perinatal depression evaluation by using self-administered questionnaires (EPDS, STAT, BDI-II, PSQI) were both performed at 26-28 week visit (2^{nd} trimester) and 6^{th} month after delivery visit. Interview for socio-demographic information, family history and maternal history was conducted at 11-13 week visit (1^{st} trimester). Anthropometric measurements including height, weight, mid upper arm circumference and skinfold, blood pressure and pulse wave velocity, eye auto-refraction, and detailed questionnaires for food diary (24-hour recall and 2-days diary), life style, family background, breastfeeding and medication intake during pregnancy were all conducted at 26-28 week visit. The maternal health records throughout full pregnancy and delivery were collected from KKH ward case notes by GUSTO coordinators. The detailed study flow is shown in **Figure 8**.

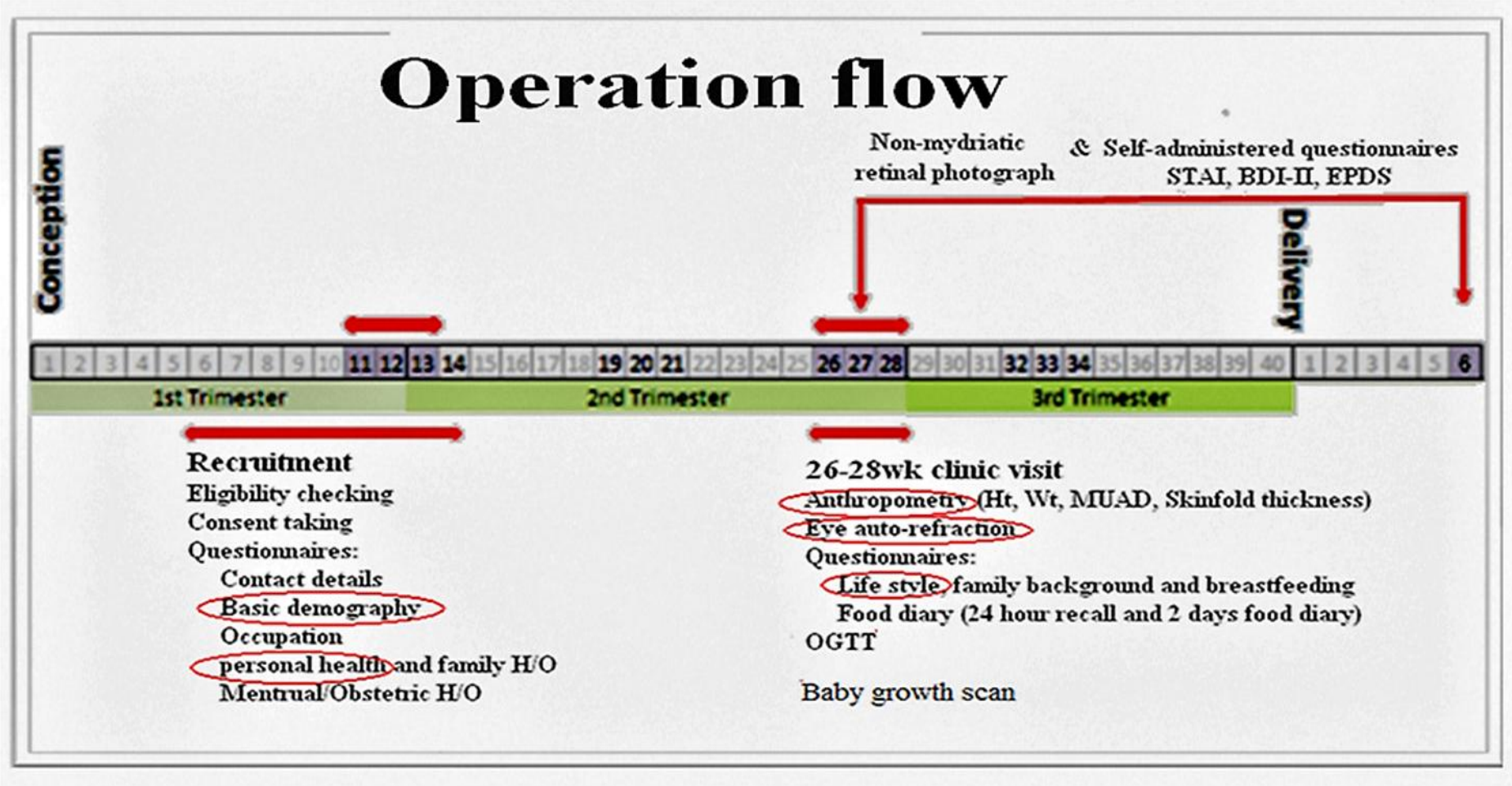


Figure 8. Clinic Operation Flow of GUSTO Retinal Study

2.7 Clinical Examinations at 26 Week's Visit

2.7.1 Blood Pressure

Blood pressure was performed on every participant and is measured by using the automatic Omron sphygmomanoter (Omron HEM 705 LP, Omron Healthcare Inc, USA). The cuff size was selected to ensure that the bladder spans the circumference of the arm and covers at least 75% of the upper arm without obscuring the antecubital fossa. The average of 3 readings was taken.

2.7.2 Anthropometric Measurements

2.7.2.1 Height

Standing height was measured from the top of the participant's head to his or her heels. To measure standing height, hair ornaments and undo braids and buns were removed. Shoes were removed as well. We asked the participant to stand erect against the backboard with the body weight evenly distributed and both feet flat on the stadiometer platform. The participants' legs were kept straight. The observer got to a face-to-face level with the subject and positioned her head so that a horizontal line was drawn from the ear canal to the lower edge of the eye socket, running parallel to the baseboard. Height was recorded to the nearest 1.0 mm. Measurements were taken in duplicate. A third measurement would be taken if the first two measurements differed by >1.0 cm. If it was necessary to take a third measurement, the two closest measurements were averaged. If the third measurement fell equally between the first two measurements, all three were averaged.

2.7.2.2 Weight

Participants were asked to remove objects such as cell phones, wallets, and toys from their pockets. In light clothing and without shoes, the participant stood in the center of the scale platform with hands at their sides and looking straight ahead. Measurements were taken in duplicate. A third measurement would be taken if the first two measurements differed by >200 grams. If it was necessary to take a third measurement, the two closest measurements were averaged. If the third measurement fell equally between the first two measurements, all three were averaged.

2.7.2.3 Mid Upper Arm Circumference

The mid-upper-arm point was half the distance between the acromion process (the most lateral bony protuberance of the back of the shoulder) and the olecranon (the bony structure that stands out when the elbow is bent). The midpoint was located for measurement of the mid-upper-arm circumference (MUAC) and triceps skinfold thickness. The participants were asked to stand upright with his/her weight evenly distributed on both feet, the right arm bent 90 degrees at the elbow, and the right palm facing up. The observer palpated the shoulder to find the acromion. The end of the spine of the right scapula was located by following the scapula out to the arm until it made a sharp V-turn to the front of the body. The observer placed the zero point of the tape on the mark over the acromion process and ran it downward along the back of the arm to the tip of the olecranon process, the bony part of the elbow. The midpoint on the posterior aspect of the arm was marked. The tape was then wrapped around the arm over the marked midpoint. The tape was positioned perpendicular to the long axis of the upper arm and was level around the circumference. The tape had

to be lied flat around and fit snugly around the arm but did not compress the skin. Two measurement were taken to the nearest 0.1 cm.

2.7.2.4 Skinfold Measurements

Measurements were taken on dry and relaxed skin on the right side of the body. The skinfold was firmly grasped by the thumb and index finger, using the pads at the tip of the thumb and finger, just 1-2 cm away from the marked site. Examiner pinched deep enough to get the fat (for a double-thick pinch of skin) but not so deep to pull up the muscle. The calipers were placed perpendicular to the fold on the site marked, at approximately 1-2cm from the finger and thumb, midway between the crest and the base, while maintaining the grasp of the skinfold. Measurement was taken 3 seconds after applying the calipers and recorded the last completed 0.2 mm. Three measurements of each skinfold site were taken and their average was used for further analysis.

Biceps- The anterior surface of the biceps midway between the anterior auxiliary fold and the antecubital fossa (anterior auxiliary line is the crease where the top of the arm, when hanging down, meets the chest). With the right arm relaxed and hanging by side and straight, the examiner grasped a vertical. While maintaining a grip on the skinfold, the examiner gently released the caliper handles and allowed the jaws to close on the fat fold for two seconds before taking the reading to the last completed 0.2 mm.

Triceps- Along the midline on the back of the triceps of the right arm, determine the midpoint located between the top of the acromial process (top of the shoulder) to the bottom of the olecranon process of the ulna (elbow). The elbow was extended and the arm was relaxed. The examiner pinched the skin to make sure the fold is running

vertically. Then she picked up the skinfold about 1 cm above the midpoint mark over the triceps muscle, with the fold running downward along the midline of the back upper arm. The caliper jaws were applied at right angles to the "neck" of the fold just below the finger and thumb over the midpoint mark. While maintaining a grip on the skinfold, the examiner gently released the caliper handles and allowed the jaws to close on the fat fold for two seconds before taking the reading to the last completed 0.2 mm.

Subscapular- The measurement point for the subscapular skinfold locating immediately below the inferior angle of the scapula was identified by palpating and marking the inferior angle of the scapula. The subject stood or sat with shoulders relaxed or gently held down to prevent movement of the scapula. The skinfold was picked up 1 cm above and medial to the subscapular mark, where the fold was taken on the diagonal line coming from the vertebral border to between 1 and 2 cm from the inferior angle of the scapulae, so that the fold ran diagonally down toward the left elbow. While maintaining a grip on the skinfold, the examiner gently released the caliper handles and allowed the jaws to close on the fat fold for two seconds before taking the reading to the last completed 0.2 mm.

Suprailiac- A diagonal fold above the crest of the ilium at the spot where an imaginary line coming down from the anterior auxiliary line. The examiner determined the anterior auxiliary line and palpated for the iliac crest (top of the hip bone). While the skin was grasped following the natural fold which followed a line of approximately from the suprailiac to just below the umbilicus (bellybutton), an angle of approximately 30 degrees, the examiner gently released the caliper handles and

allowed the jaws to close on the fat fold for two seconds before taking the reading to the last completed 0.2 mm.

2.7.3 Eye Examinations

2.7.3.1 Fundus Photography

Right eye of each participant was taken without pupil dilation. The procedure is the same as what has been introduced in the STARS and STARS Family retinal study. At least three retinal photos were taken on field 1, field 2 and field 3.

2.7.3.2 Auto-refraction

Non-cycloplegic autorefraction was only performed among all participants with retinal photographs taken during the 26-28 week visit by using the Canon Autorefractor RK-F1 (Canon, Tokyo, Japan). A total of 5 consecutive readings were obtained. The autorefractor readings were acceptable if the difference between the lowest and highest reading was 0.25 diopters or less.

2.8 Retinal Vessel Assessment

2.8.1 Training Program

This training program for Singapore I Vessel Assessment (SIVA version 3.0) was arranged as a 3-month course in Fushionopolis, Singapore.

2.8.2 Training Assessment

10% of the graded retinal photos were graded again by the same grader. Major retinal vasculature parameters including retinal vascular caliber, retinal vascular tortuosity, retinal vascular branching angle, retinal vascular branching coefficient, and retinal fractal dimension were tested for intra-grader reproducibility. The correlation

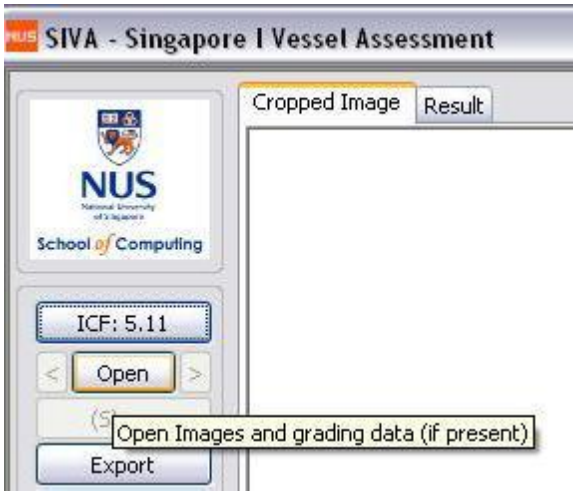
of 80% and above for each retinal vascular parameter was considered as valid and reliable.

2.8.3 Retinal Grading System

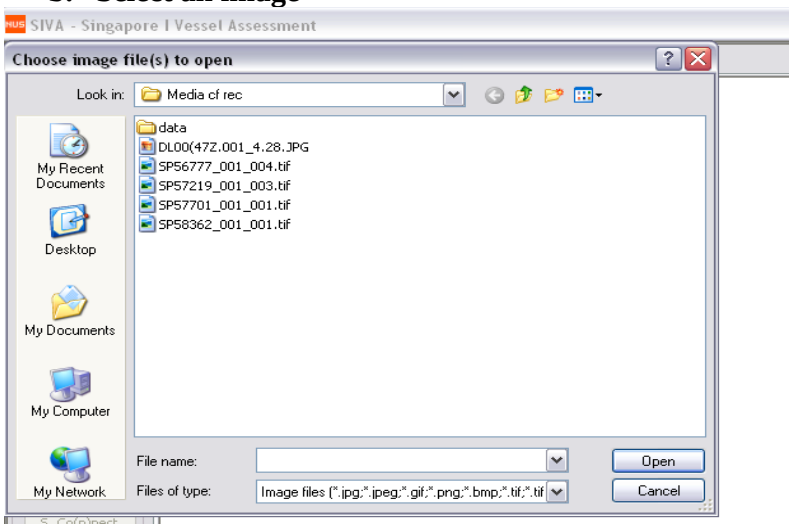
The grading system of retinal photographs of GUSTO retinal study was done according to Singapore I Vessel Assessment Revised Protocol (SIVA 3.0). Before grading an image, Field 1 (Optic disc centered) images with minimum resolution (3000x2000 pixels) in JPG or TIFF images were basically required.

2.8.3.1 Operation Procedures

a. Click on "OPEN"



b. Select an image

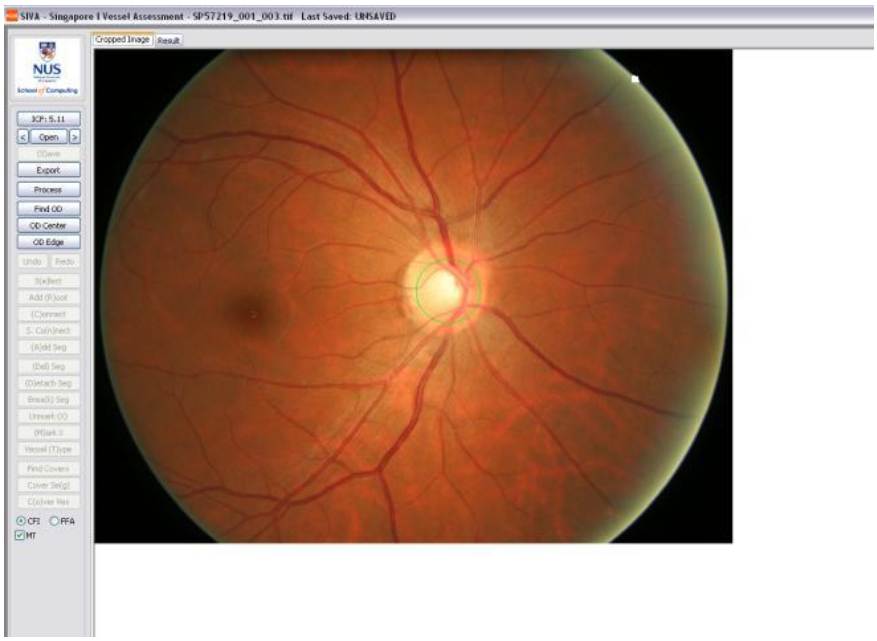


c. Let the image load and once it is loaded, click on "Find OD" first.

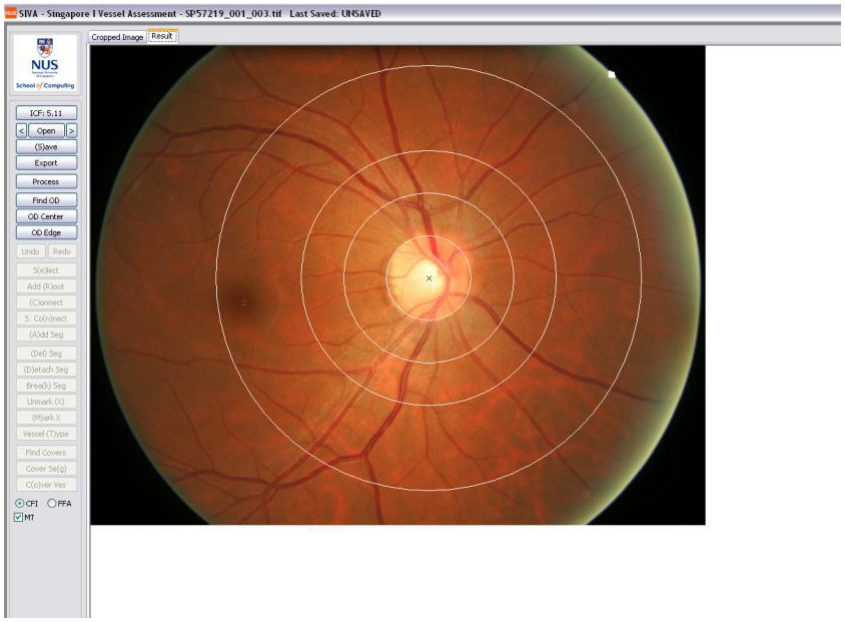
d. Find OD Method:

In normal cases, optic disc was automatically detected. Then we clicked on "Process" to locate the vessels.

OD Centre Method: If the optic disc was not detected accurately, we clicked on OD Center. The circle was placed directly over the cup. Once locating the cup, we clicked on the image. The circle outlined the boundary of the cup. Once satisfied, we clicked on "Re-process" and the optic disc and the vessels were detected.



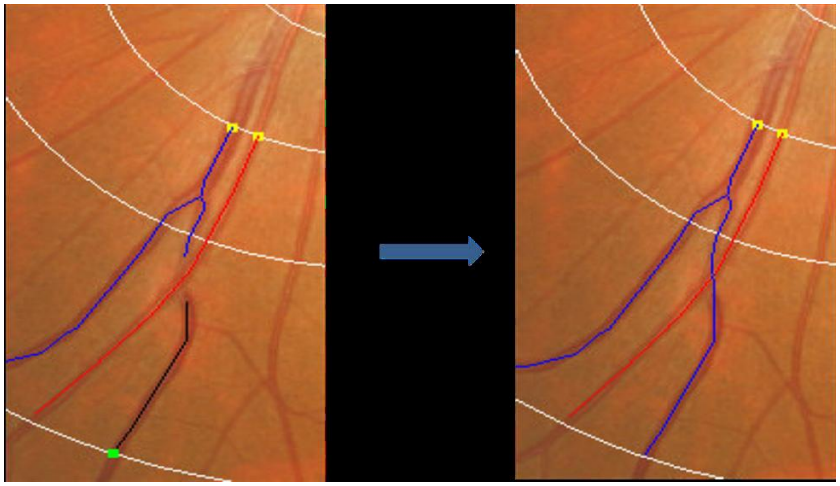
OD Centre & Edge Method: For special cases where the "Find OD" method or the "OD Centre" method fails to detect the optic disc satisfactorily, the old SIVA method of detecting the optic disc was used. We firstly clicked on the "OD Centre and located the centre of the cup and then clicked on "OD Edge" and brought the mouse pointer to the edge of the optic disc. When the program detected the optic disc, we clicked "Process" to detect the vessels.



e. Identify the traced vessels as arterioles or venules

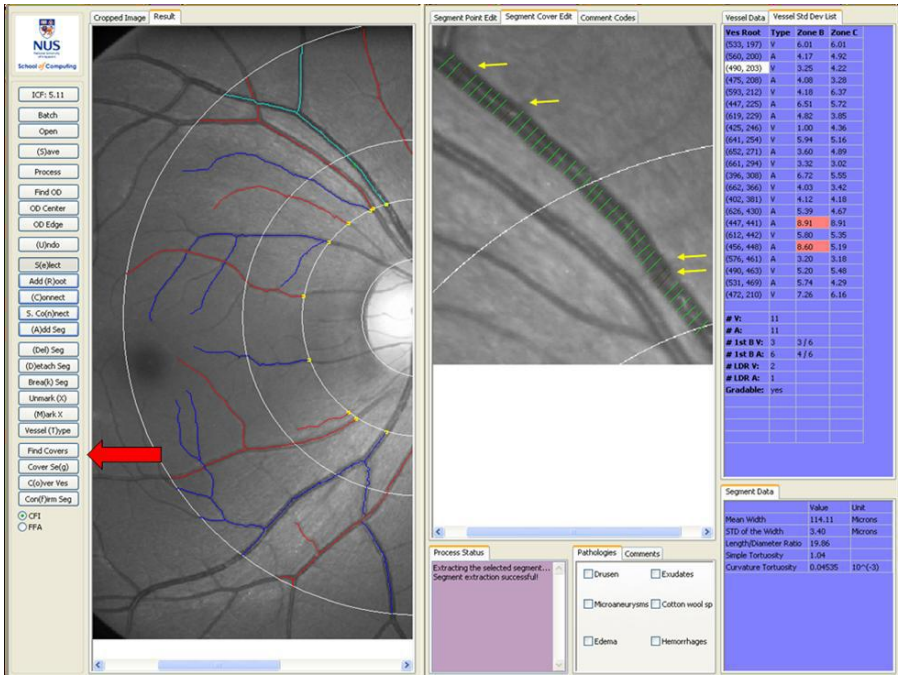
To change the vessel type, we clicked on "Vessel Type". Arterioles were usually straight, less tortuous. They were thinner and paler in color compared to veins. Venules were usually more tortuous, thicker and darker in color.

The end of Zone C was traced all the way up (only trace up to the points which the Grader is confident upon, if not let SIVA decide). Manual tracing should be avoided as much as possible. The Black & White or Red-Free filter were used to get a better view. The full length of the vessel was traced and any broken segment was joined by using "Connect" on the 2 end of the broken segments.



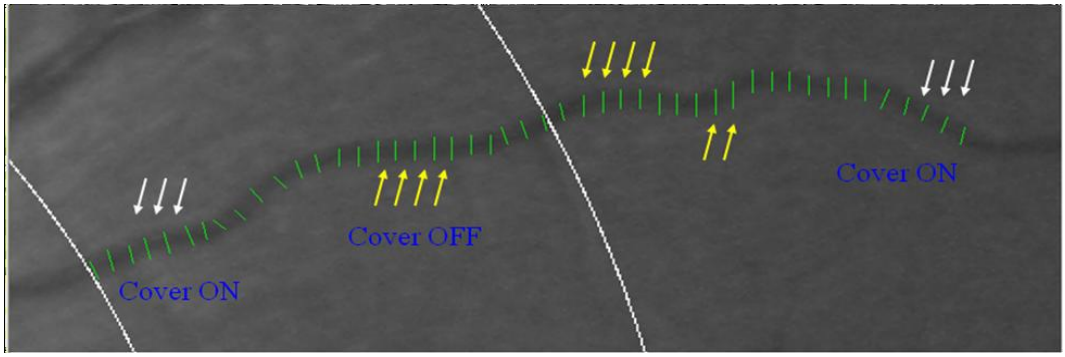
f. Find covers

After all the vessels had been traced, we used the "Find Covers" to toggle automatic detection of irregular covers and switch them off.



g. Use covers to switch ON and OFF ONLY.

A minimum of 5 covers were needed, no maximum number. We needed to accept that at times SIVA will take on "shadows" but not when the covers were twice the width size. When there were vessels with irregular widths i.e venous beading, covers of different parts of the vessel were used.

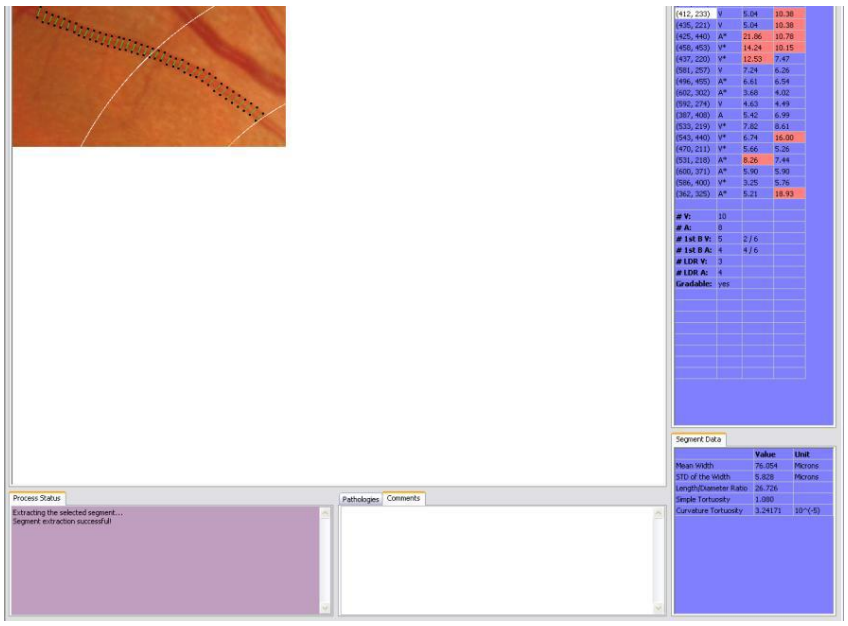


h. Edit segment

We used “edit segment” only if all the covers were wrong, by selecting a few varied points in the vessel such as start, mid-point and end as long as Grader was confident of the visibility of the vessel wall.

i. Check all the vessels are done and save data.

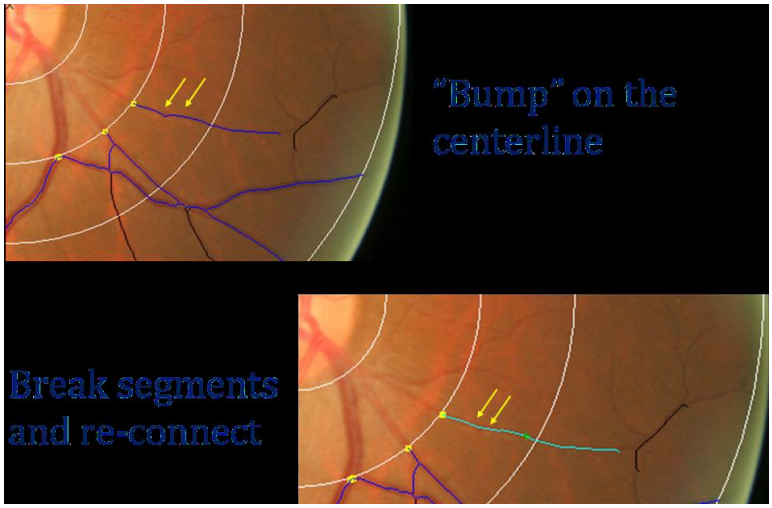
Comments were entered in the comments box which was reflected in exported excel sheet containing all the information pertaining to the image, ie. vessel width, tortousity etc.



2.8.3.2 Special notification during grading

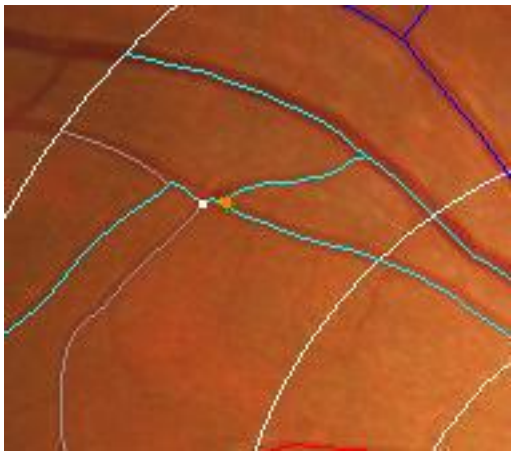
a. Bumps:

If centerline tracing was not smooth, the bump was removed and the tracing was reconnected.



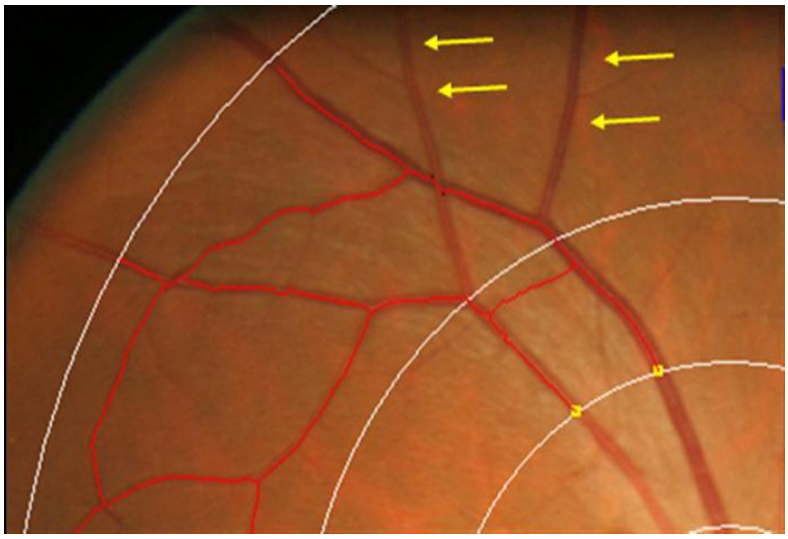
b. Crossovers

If the crossover was not automatically detected or it was a wrong crossover, we clicked on "Mark X" to mark the point of crossover between the two vessels. To remove crossovers, we clicked on "Unmark X" at the point of intersection. The vessels which might be discontinuous were connected together by using the "Connect" button.



c. Branching

Branching vessels which had been traced by SIVA (include the 2nd branches) should be drawn and ensured that segments were accurate.



d. Reliability Test of Retinal Vessel Assessments

A single grader, masked to blood pressure measurements and participant characteristics, performed all of the retinal vascular caliber measurements for this cohort. Intra-grader reliability was assessed in 90 randomly selected retinal photographs from the GUSTO cohort, and the intraclass correlation coefficient was 0.94 for retinal arteriolar caliber, 0.97 for retinal venular caliber, 0.821 for retinal branching angle, 0.92 for fractal dimension, 0.95 for retinal arteriolar tortuosity, and 0.87 for retinal venular tortuosity.

2.9 Interview at the Clinic

Interviews were conducted during the clinic visit. The questionnaire was designed in English and translated into Chinese, Malay and Tamil three languages. The interviews were conducted by a study research assistant and took approximately 30-60 minutes to complete. Questionnaires were administered to capture family history, maternal smoking, alcohol, drug use, medical history, physical activity, and use of traditional medicines and supplements.

2.9.1 First Clinic Visit (11-12 week)

Demographic factors such as age, gender, total family income, housing and household composition, patient and her partner's completed educational level and occupation were collected. Personal health such as asthma, hypertension, type 2 diabetes, myopia, menstrual cycle and pregnancies, and relevant medication intake were collected. Family history such as genetic disease, chronic disease and tumor were provided by the participants. Household income was classified into five categories as follows: 1. SGD 0-999 per month; 2. SGD 1,000-1,999 per month; 3. SGD 2,000-3,999 per month; 4. SGD 4,000-5,999 per month; 5. More than or equal to SGD 6,000 per month. Pregnancy outcome history was classified into 2 categories as follows: 1. No live birth given before; 2. At least one live birth given.

2.9.2 Second Clinic Visit (26-28 week)

A series of information before pregnancy and after pregnancy such as activity and exercise, alcohol consumption and smoking were collected. Personal views on breastfeeding, medication during pregnancy, contraception use before the latest gestation, digestive symptoms and dieting changes during pregnancy, pregnancy

complication such as bleeding, hospitalization and relevant medication were subsequently asked. Evaluation of maternal mental status was done by self-administered questionnaires as follows: STAI (State Trait Anxiety Inventory), BDI-II (Beck Depression Inventory Second Edition) and EPDS (Edinburgh postnatal depression scale).

2.10 Delivery Record

Delivery information was collected from KKH ward records. Delivery report form reported all pregnancy complications such as gestational hypertension, gestational diabetes, pre-eclampsia, IUGR (Intrauterine Growth Restriction), multiple pregnancy, pre-term babies and neonatal problems. Investigation sheets reported all pregnancy laboratory examinations such as full blood count, HBsAg, HBsAb, HIV test and high vaginal swab.

2.11 Clinical Definitions

2.11.1 Gestational Hypertension

Gestational hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg in a previously normotensive pregnant woman who is ≥20 weeks of gestation and has no proteinuria.

2.11.2 Gestational Diabetes

Gestational diabetes (or gestational diabetes mellitus, GDM) was a condition in which women without previously diagnosed diabetes exhibit high blood glucose levels during pregnancy. According to WHO guideline for gestational diabetes, patients with gestational diabetes had abnormal oral glucose tolerance test (OGTT),

which was fasting level of serum glucose between 6.1 and 7.0 mmol/l (110 and 125 mg/dl) and 2 hour OGTT glucose level between 7.8 mmol/l and 11.1 mmol/l (140 and 200 mg/dl).[254]

2.11.3 Pre-eclampsia

Pre-eclampsia was a medical condition in which hypertension arises in pregnancy (pregnancy-induced hypertension) in association with significant amounts of protein in the urine.[255] Pre-eclampsia was diagnosed when a pregnant woman develops high blood pressure (two separate readings taken at least 6 hours apart of 140/90 or more) and 300 mg of protein in a 24-hour urine sample (proteinuria).[255] A rise in baseline blood pressure (BP) of 30mmHg systolic or 15mmHg diastolic, while not meeting the absolute criteria of 140/90, was still considered important to note, but was not considered diagnostic. Hypertension and proteinuria were necessary for a diagnosis of pre-eclampsia.[255]

2.11.4 High Risk for Pre-eclampsia

A recent meta-analysis had reported that MABP in the second trimester was a better predictor of preeclampsia than SBP or DBP, with a predictive strength of MABP 90mm Hg as moderate (area under the receiver operating characteristic curve, 0.76).[256, 257] We, therefore, defined pregnant women in our study who had MABP 90mm Hg as “high” risk in developing incident preeclampsia and those with MABP 90mm Hg as “low” risk.

2.11.5 Maternal Obesity

According to World Health Organization prepregnancy BMI criteria, we classified patients into four groups as follows: underweight (less than 18.5), normal weight (18.5–24.9), overweight (25.0 –29.9), obese (30.0 or greater).[74] We chose to use these rather than the lower Asian standards used by Jafar et al[258] (overweight or obese as 23.0 or greater) to allow comparison to available literature and guidelines.

2.11.6 Weight Gain

Full-term BMI-specific weight gain cutoff was referred to the Institute of Medicine weight gain guideline. As recommended by the Institute of Medicine, ideal weight gain was 12.5–18 kg, 11.5–16 kg, 7.0 –11.5 kg, and less than 7.0 kg for underweight, normal weight, overweight, and obese women based on their pre-pregnancy BMI, respectively.[259] Because there was no guideline for ideal weight gain at 26 weeks of gestation or the second trimester, we used the Institute of Medicine guideline as a reference; thus, three groups (less than ideal weight gain, within ideal weight, and greater than ideal weight gain) were classified accordingly among our patients.

2.11.7 Mental Health

Symptoms of antenatal depression were measured with the Edinburgh Postnatal Depression Scale (EPDS).[260] Symptoms of antenatal anxiety were measured with the State-Trait Anxiety Inventory (STAI),[261] only the State anxiety subscale was used for the analyses. Antenatal sleep quality was measured with the Pittsburg Sleeping Quality Index (PSQI). [262] [256] [252242] [258] [25816] All three assessments were taken at the 26 weeks visit. The EPDS is a widely used self-administered 10-item screening method

for depression in the postnatal period. It has proven to be reliable and sensitive in detecting perinatal depression and has also been used to screen antenatal depression in pregnant women. [260] [254] [250240] [256] [25614] Based on Spitzer's Research Diagnostic Criteria, a cut-off of 13 and 14 is generally used as its prediction on possible postnatal depression and possible antenatal depression for most women, respectively. [263] [257] [253243] [259] [25917]

The STAI consists of 40 questions with two subscales, state-anxiety and trait-anxiety, and each summated separately. It is a widely used and accepted measure for anxiety in adults. It differentiates between temporary condition of "state anxiety" (a temporary acute anxiety state) and the more general and long-standing quality of "trait anxiety" (a propensity for general anxiety). In our study, STAI raw scorings and categorized scores were analyzed. Cut-off for high STAI score was determined by top 75 percentile suggested by Nasreen et al and Teixeira et al.[264]

The validated PSQI consists of 19 self-rated questions to assess a wide variety of factors relating to sleep quality and the frequency and severity of specific sleep-related problems. The PSQI has seven subscales each of a score of 0-3, which together adds up to a total PSQI score with a range of 0-21.[262] Participants with PSQI total score 6 and above are considered as poor sleep quality.[262]

2.12 Statistical Analysis

The student's t-test and χ^2-test were used to compare means/categorized data of potential variables of interest between group with retinal photography and group without retinal photography according to our outcomes. If the variables of interest were categorized into more than two groups (Ethnicity, Household income), the differences among the groups were analyzed by using one-way ANOVA test. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated when the outcomes are generalized retinal arteriolar narrowing and generalized retinal venular widening.

Independent variables of interests as blood pressure measurement (SBP, DBP and MABP), anthropometric measurements (weight, height, mid-upper arm circumference, subcutaneous skinfold thickness and BMI) and antenatal mental health assessment (EPDS, STAI and PSDI scores) were all analyzed continuously and categorized into quintiles. All these variables were further classified into quartiles or quintiles or groups by respective clinical cut-offs.

Dependent variables as retinal arteriolar and venular caliber, retinal arteriolar and venular tortuosity, retinal arteriolar and venular branching angle and retinal arteriolar and venular fractal dimension were estimated based on two models: model 1: age and ethnicity-adjusted; and model 2: multivariate-adjusted. Since retinal venular caliber accounts for approximately 30% of the variability in arteriolar caliber as a result of shared genetic and ocular factors and vice versa, retinal fellow vessels were taken into account for major confounders. Backward stepwise modeling was conducted to determine the best-fitting and most parsimonious model. Test of trend was

determined by treating quintiles and clinical categories of all independent variables as continuous variables in ordinal scale. Multiple regression models were used to estimate the changes in retinal vascular caliber with every SD increase in major variables.

Potential interactions of each variable by ethnicity, household income, etc. were examined in stratified analyses and as interaction terms in the models.

Finally, retinal vascular parameters were used as determinant and gestational complications including gestational hypertension, pre-eclampsia and gestational diabetes were used as outcomes. The relationships between retinal vascular parameters and gestational complications were calculated into multiple logistic regression odds ratio. A significant p value (two-tailed) was defined as <0.05. All statistical analyses were performed using PASW 19.0 (SPSS Inc).

Chapter 3

3. Results

3.1 Basic Characteristics of the Study Population

A. STARS and STARS Family retinal study

Table 26 compares basic characteristics including age, gender, parental demographic status, parental life style, blood pressure, anthropometric measurement and ocular assessments among boys and girls who were recruited in our children retinal study. In our 586 Singapore Chinese children, the proportions of boys and girls are nearly equal to each other. For most of the factors such as age, gender, father's education, parental life style, blood pressure and anthropometric measurements, there was no significant difference between boys and girls.

However, girls tended to have a smaller birth weight, shorter birth length, a set of smaller ocular biometric parameters than boys but not smaller retinal vascular calibers. The means of retinal arteriolar caliber and retinal venular caliber were higher in girls (161.07±15.44 vs. 155.34±15.15, $p<0.01$; 224.17±21.43 vs. 218.80±19.68, $p<0.01$) than boys.

Table 26. Basic Characteristic between Boys and Girls among Children with Retinal Photography Participated in STARS and STARS Family Retinal Study

Variables	N	Mean (SD) or number (%) Boys	Girls	p*
Age, years	586	6.23 (2.78)	6.43 (2.84)	0.38
Gender, %	586	289 (49.4%)	297 (50.6%)	--
Demographic factors:				
Father's Education, %	454	230 (50.7%)	224 (49.3%)	0.83
≤Secondary school,	135	70 (51.9%)	65 (28.1%)	--
O/N level,	85	40 (47.1%)	45 (52.9%)	--
A levels/diploma,	107	57 (53.3%)	50 (46.7%)	--
University education,	127	63 (49.6%)	64 (50.4%)	--
Parental life style:				
Mother smoking history, Yes	32	18 (56.3%)	14 (43.7%)	0.39
Father smoking history, Yes	174	82 (47.1%)	92 (52.9%)	0.48
Mother smoking during pregnancy, Yes	8	2 (25%)	6 (75%)	0.13
Blood pressure measurement:				
Systolic blood pressure, mmHg	514	103.54 (12.70)	102.40 (12.01)	0.30
Diastolic blood pressure, mmHg	514	62.31 (7.88)	63.29 (9.01)	0.19
Mean arterial pressure, mm Hg	514	76.05 (8.54)	76.32 (9.18)	0.73
Anthropometric measurement:				
Body mass index, kg/m^2	586	16.30 (2.95)	16.01 (2.54)	0.19
Triceps skinfold thickness, mm	525	11.42 (3.90)	11.93 (3.34)	0.11
Birth parameters:				
Birth weight, kg	435	3.13 (0.46)	3.02 (0.47)	<0.01
Birth length, cm	431	49.39 (2.11)	48.73 (2.41)	<0.01
Head circumference, cm	430	33.52 (1.40)	33.31 (1.68)	0.16
Gestational age, wks	89	38.81 (1.20)	38.11 (2.58)	0.09
Ocular biometric parameters:				
Right eye spherical equivalent, diopter	586	-0.13 (2.10)	-0.08 (2.21)	0.77
Right eye axial length, mm	454	22.59 (0.75)	22.06 (0.68)	<0.01
Right eye anterior chamber depth, mm	430	3.40 (0.23)	3.33 (0.23)	<0.01
Right eye corneal Curvature, mm	411	7.77 (0.25)	7.64 (0.21)	<0.01
Right Eye Retinal vascular caliber:				
CRAE, μm	586	155.34 (15.15)	161.07 (15.44)	<0.01
CRVE, μm	586	218.80 (19.68)	224.17 (21.43)	<0.01
AVR	586	0.71 (0.07)	0.72 (0.07)	0.16

*Student's t-test or χ^2 test

B. GUSTO retinal study

Table 27 shows basic characteristics between pregnant women with retinal photography and those without retinal photography who were recruited in GUSTO main study and GUSTO IVF study. For most of the major variables, women with retinal photographs taken were comparable to those without retinal photographs taken, only that women in our retinal study tended to smoke more cigarettes and drink more alcohol before or during their pregnancy.

According to the basic characteristics, the mean age of our participants was 30.51 years, with ethnic composition of Chinese, Malay and India as 5:3:2. There was only a small amount with hypertension and diabetes history (both less than 3%), and the average mental health status tended to be normal (EPDS, BDI and PSQI were below clinical cut-off).

Table 27. Basic Characteristic between Pregnant Women with Retinal Photography and without Retinal Photography Participating the GUSTO Main and the GUSTO IVF Study

	Mean (SD) or sample number (%)				
Variables	**N**	**With retinal photography**	**N**	**Without retinal photography**	**p***
Age	824	30.51 (5.38)	128	30.58 (5.92)	0.28
Ethnicity, %	--	--	--	--	0.47
Chinese	450	54.6%	63	49.6%	--
Malay	235	28.5%	38	29.9%	--
India	139	16.9%	27	20.5%	--
IVF, Yes %	67	8.1%	5	4.0%	0.12
Primiparous, Yes %	420	51.0%	65	50.9%	0.96
Demographic Factor:					
Household income,	--	--	--	--	0.80
1. SGD 0-1999 per month	162	19.7%	28	21.4%	--
2. SGD 2000-3999 per month	280	34.0%	31	23.9%	--
3. SGD 4000-5999 per month	209	25.3%	32	24.8%	--
4. >=SGD 6000 per month	173	21.1%	37	21.4%	--
Medical history:					
Hypertension history, Yes %	19	2.3%	2	1.5%	0.81
Diabetes history, Yes %	8	1.0%	2	1.5%	0.88
Life style:					
Cigarette smoking history, Yes %	122	14.9%	12	9.4%	<0.01
Alcohol intake history, Yes %	255	30.9%	33	25.8%	<0.01
Blood pressure measurement:					
Systolic blood pressure, mm Hg	665	111.17 (12.38)	13	104.74 (11.31)	0.06
Diastolic blood pressure, mm Hg	665	67.09 (9.36)	13	68.19 (12.95)	0.68
Mean arterial pressure, mm Hg	665	81.78 (9.11)	13	80.37 (9.87)	0.58

Variables	N	Mean (SD) or sample number (%) With retinal photography	N	Without retinal photography	p*
Anthropometric measurement:					
Height, cm	824	157.97 (7.85)	60	158.46 (5.18)	0.63
Pre-pregnancy weight, kg	741	57.28 (12.17)	50	59.30 (12.00)	0.67
Weight at 26 weeks, kg	814	65.76 (12.81)	60	67.28 (11.57)	0.37
Weight gain at 26 weeks, kg	741	8.59 (4.86)	50	9.83 (5.16)	0.07
Pre-pregnancy BMI, kg/m^2	741	22.96 (4.64)	50	22.93 (4.74)	0.97
26 week's BMI, kg/m^2	814	26.36 (4.57)	60	27.05 (4.85)	0.27
Body fatness assessment:					
Mid-upper arm circumference, mm	813	28.18 (4.19)	75	28.81	4.20
Triceps skinfold, mm	813	22.90 (6.06)	75	24.04 (6.18)	0.12
Biceps skinfold, mm	814	12.53 (5.39)	75	13.38 (5.78)	0.20
Subscapular skinfold, mm	813	22.54 (6.50)	75	24.11 (6.98)	0.05
Suprailiac skinfold, mm	812	23.67 (5.59)	75	24.48 (6.21)	0.24
Refraction:					
Right eye spherical equivalent, D	824	-2.28 (2.67)	117	-1.93 (1.85)	0.65
Antenatal mental health assessment:					
EPDS					
1. EPDS depression score	824	3.70±1.95	65	3.42±1.85	0.25
2. EPDS anxiety score	824	4.38±3.15	65	4.05±2.83	0.42
3. EPDS total score	824	8.08±4.49	65	7.46±4.19	0.29
BDI-II	817	8.83±6.64	63	8.19±6.13	0.46
STAI					
1. State Anxiety score	801	35.63 (9.88)	63	34.22 (9.72)	0.27
2. Trait Anxiety score	801	37.40 (9.17)	63	36.23 (9.76)	0.32
3. STAI total score	801	72.54 (18.26)	63	70.45 (18.82)	0.37
PSQI	483	5.71 (2.90)	39	5.73 (3.10)	0.98
Oral glucose tolerance test (OGTT):					
Fasting glucose level	701	4.39 (0.49)	120	4.50 (0.31)	0.56
2-hr glucose level	701	6.50 (1.47)	120	6.71 (1.76)	

Abbreviation: SD, standard deviation; BMI, body mass index; EPDS, Edinburgh Postnatal Depression Score; BDI-II, Beck Depression Inventory second version; STAI, State-Trait Anxiety Inventory; PSQI, Pittsburgh Sleeping Quality Index.
* Student's t-test or χ^2 test

3.2 Distributions of Potential Variables of Interest

3.2.1 Distributions of Blood Pressure Measurements

A. STARS and STARS Family retinal study **B. GUSTO retinal study**

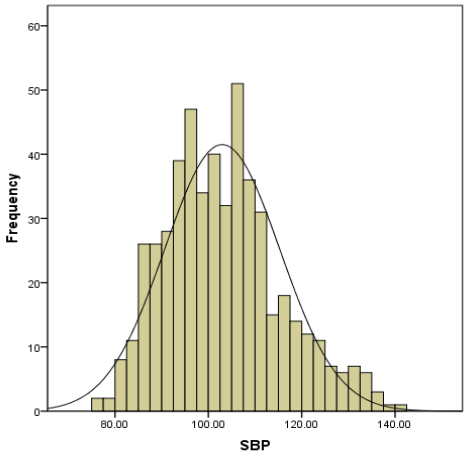


Figure 9A.

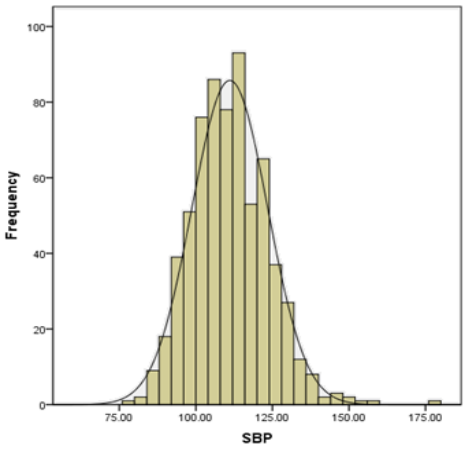


Figure 9B.

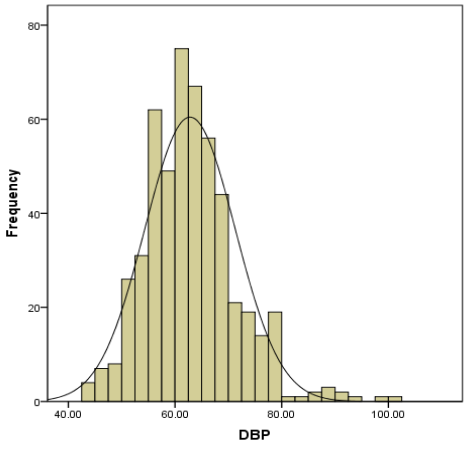


Figure 9A.

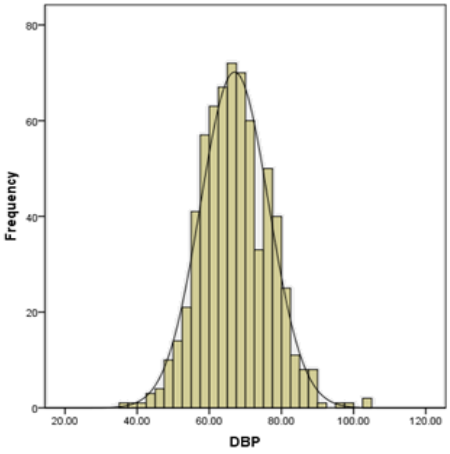


Figure 9B.

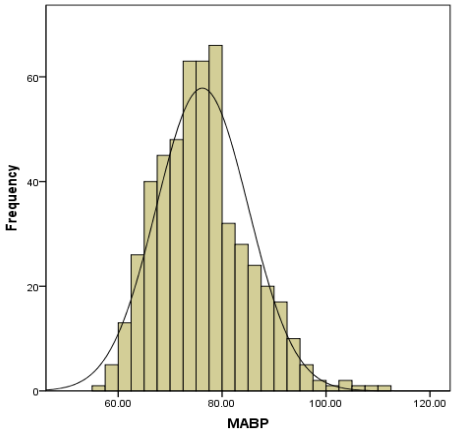


Figure 9A.

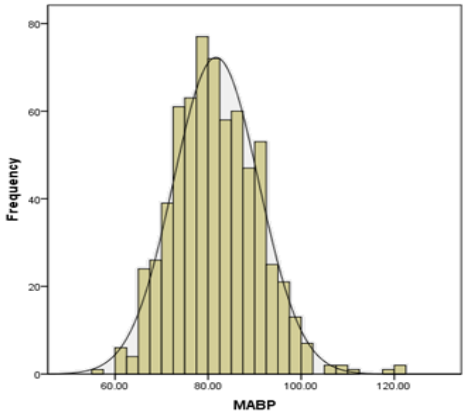


Figure 9B.

All blood pressure measurements in children and pregnant women were normally distributed. The distributions of systolic blood pressure (SBP), diastolic blood pressure (SBP) and mean arterial blood pessure (MABP) in children were shown in Figure 9A while these distributions in pregnant women were shown in Figure 9B. The skewness and kurtosis of SBP are 0.51 and -0.04 in children and 0.37 and 0.33 in Pregnant women, respectively. The skewness and kurtosis of DBP are 0.80 and 1.71 in children and 0.24 and 0.45 in Pregnant women, respectively. The skewness and kurtosis of MABP are 0.63 and 0.65 in children and 0.39 and 0.57 in pregnant women, respectively.

3.2.2 Distributions of Anthropometric Measurements

A. STARS and STARS Family retinal study

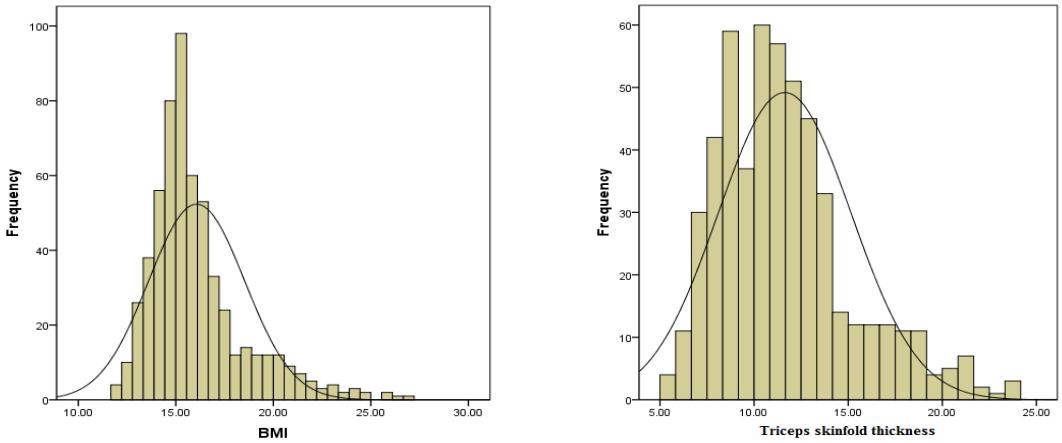
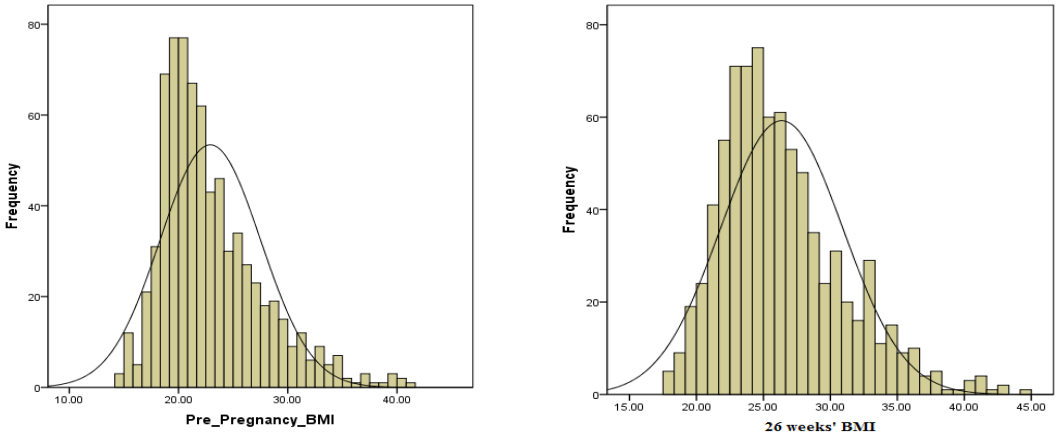


Figure 10A.

The distributions of body mass index (BMI) and triceps skinfold thickness (TSF) in children are shown in **Figure 10A**. The skewness and kurtosis were 2.30 and 9.32 in BMI and 1.01 and 1.20 in TSF, respectively.

B. GUSTO retinal study



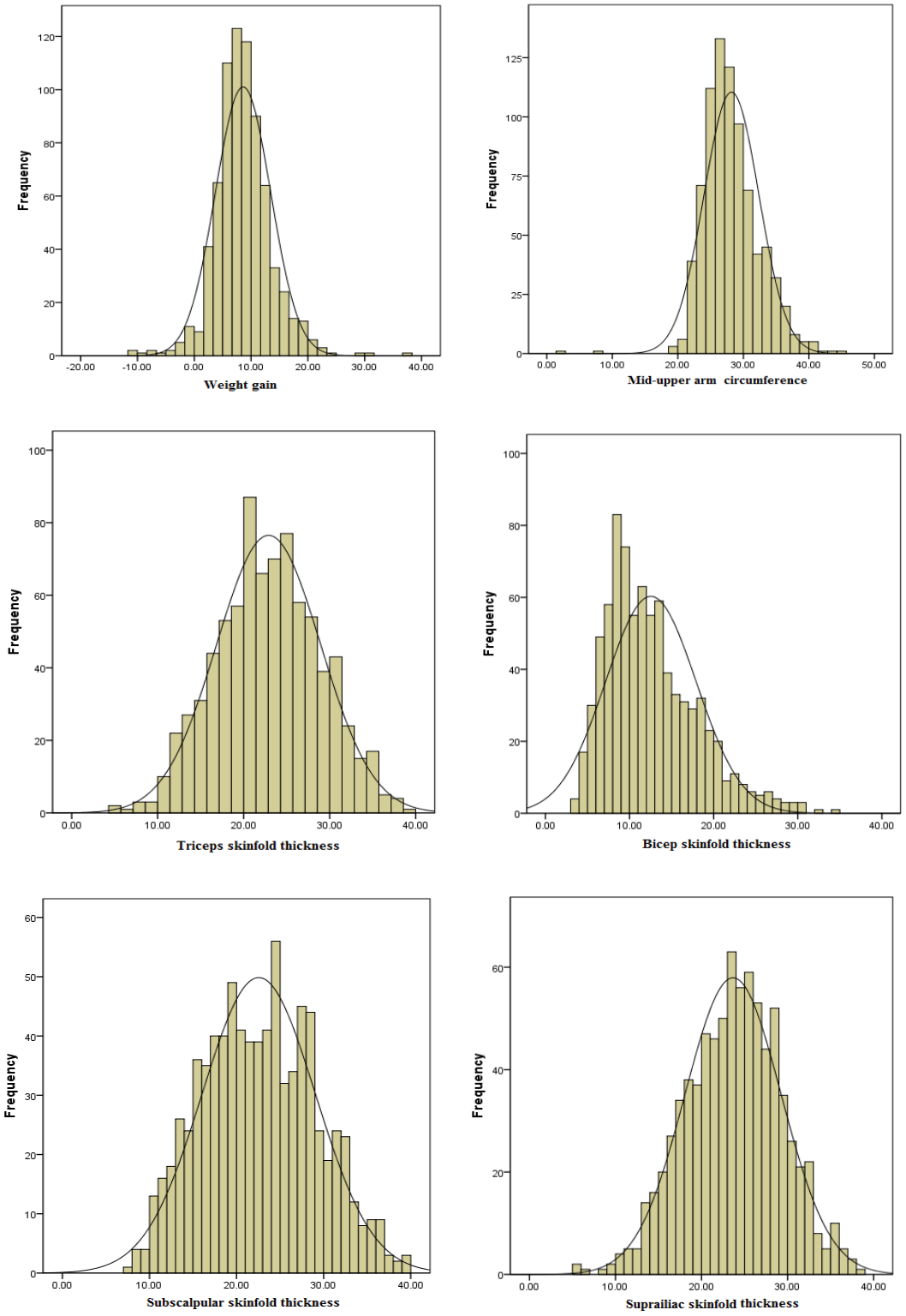

Frequency
Weight gain
Mid-upper arm circumference
Triceps skinfold thickness
Bicep skinfold thickness
Subscalpular skinfold thickness
Suprailiac skinfold thickness

Figure 10B.

The distributions of pre-pregnant BMI, 26 week's pregnant BMI, weight gain, mid-upper arm circumference (MUAC), four types of subcutaneous skinfold thicknesses were shown in Figure 10B. The skewness ranged from -0.14 to 1.61 and the kurtosis ranged from -0.61 to 3.39 across all anthropometric variables in pregnant women.

3.2.3 Distributions of Refraction and Ocular Biometric Parameters

A. STARS and STARS Family retinal study

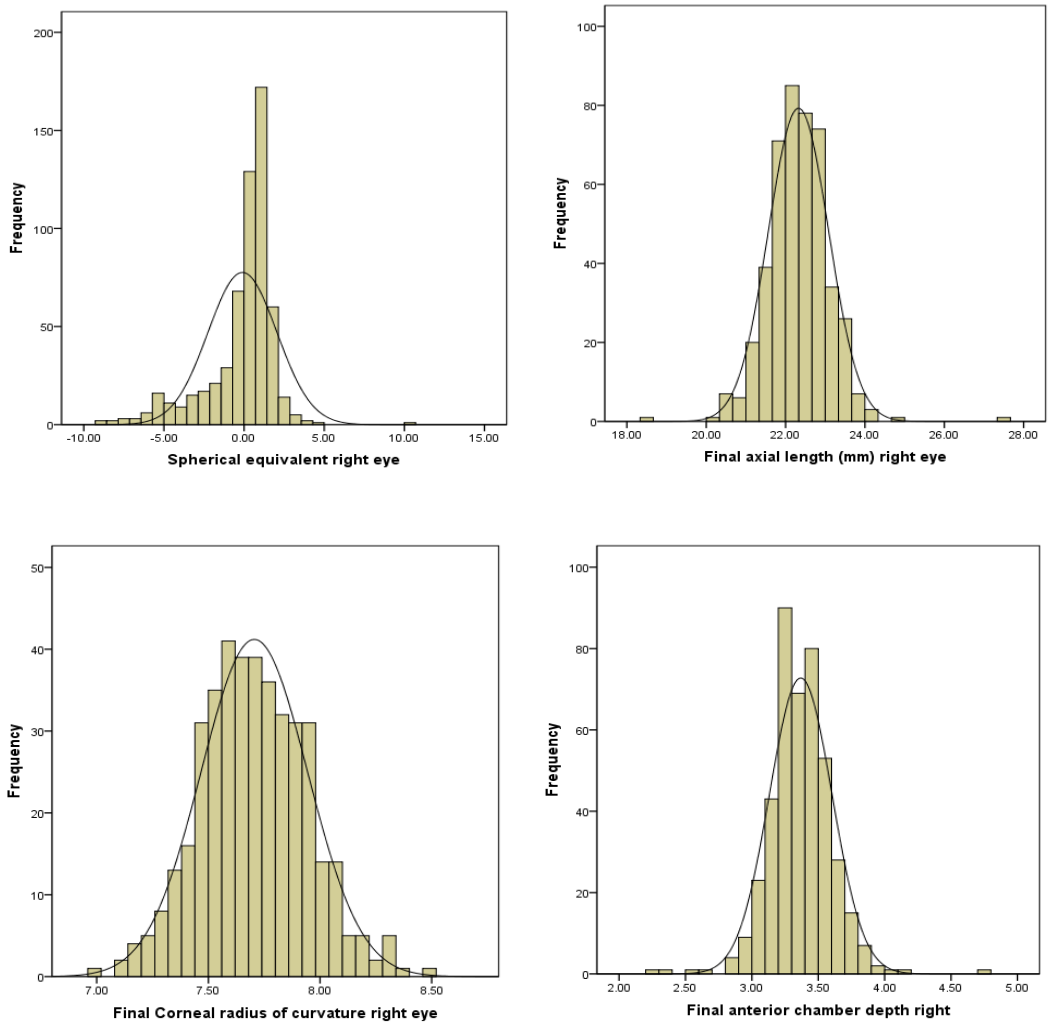


Figure 11A.

The distributions of spherical equivalent (SE), axial length (AL), corneal curvature (CC) and anterior chamber depth (ACD) of right eyes in children were shown in **Figure 11A**. The skewness ranged from -1.37 to 0.35 and the kurtosis ranged from 0.10 to 5.20 across all anthropometric variables in children.

B. GUSTO retinal study

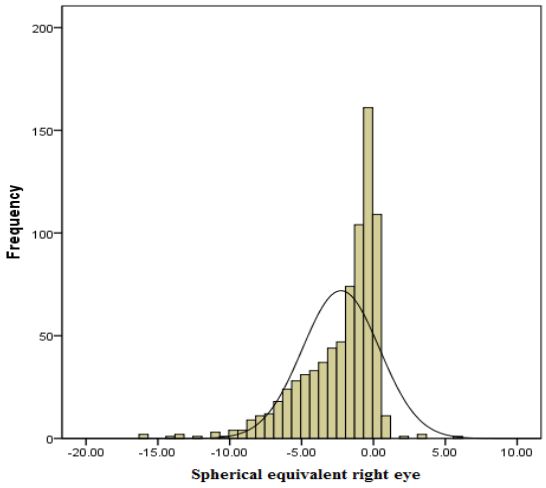


Figure 11B.

The distributions of right eye spherical equivalent in pregnant women were shown in **Figure 11B**. The skewness and kurtosis were -1.47 and 2.89, respectively. The distribution of SE in our pregnant subjects was skewed to the left.

3.2.4 Distributions of Mental Health Assessment

GUSTO retinal study only

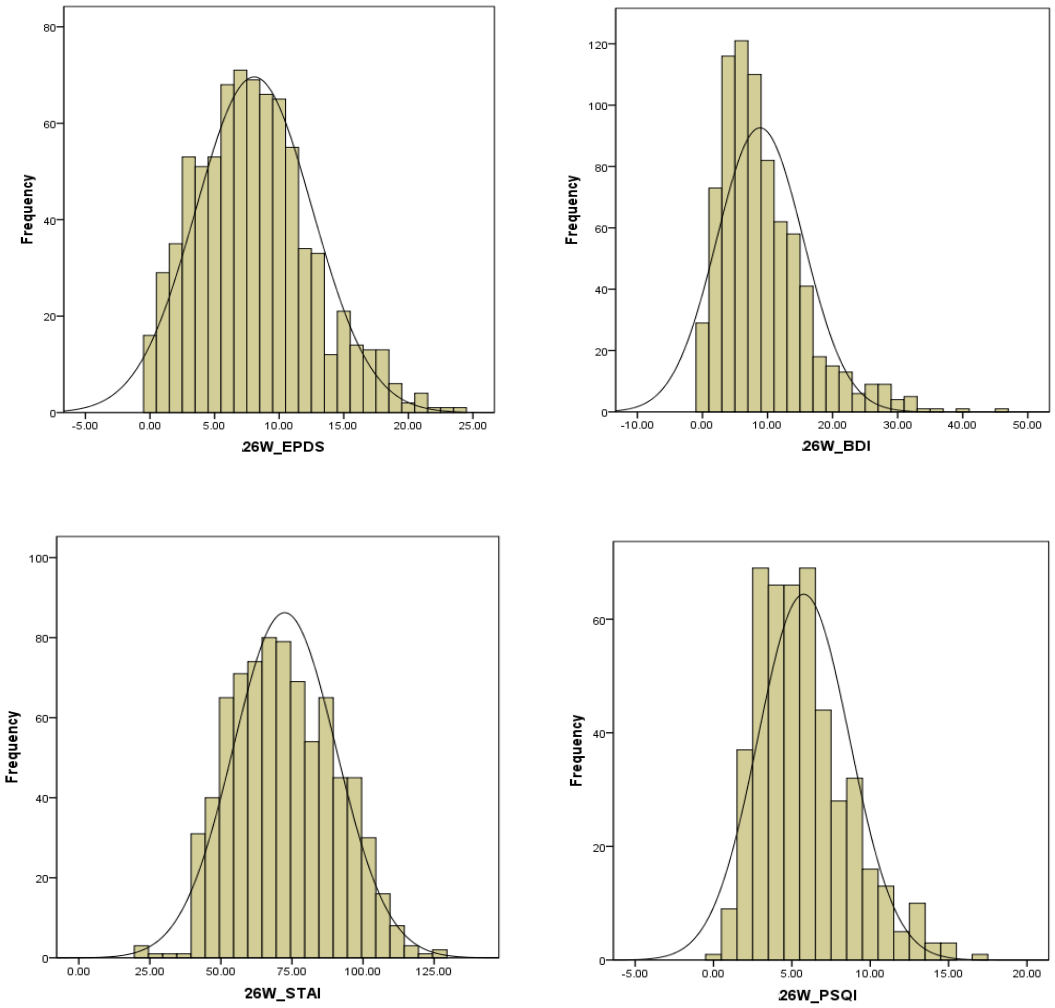


Figure 12.

The distributions of Edinburgh Postnatal Depression Score (EPDS), Beck Depression Inventory second version (BDI-II), State-Trait Anxiety Inventory (STAI) and Pittsburgh Sleeping Quality Index (PSQI) are shown in Figure 13. The skewness and kurtosis of each mental health assessment score were 0.20-1.42 and -0.47-2.82, respectively.

3.2.5 Distributions of Birth Parameters

STARS and STARS Family retinal study only

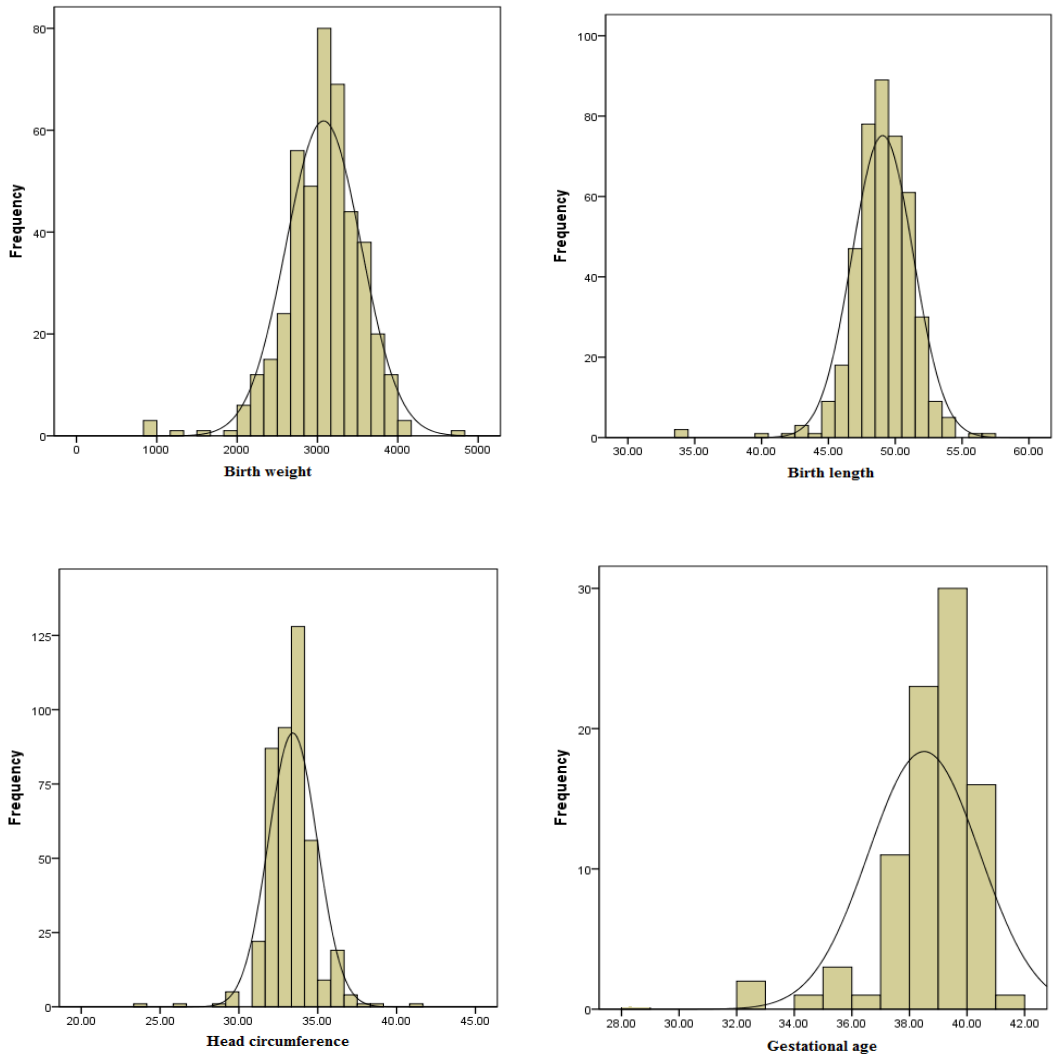


Figure 13.

The distributions of Edinburgh Postnatal Depression Score (EPDS), Beck Depression Inventory second version (BDI-II), State-Trait Anxiety Inventory (STAI) and Pittsburgh Sleeping Quality Index (PSQI) are shown in Figure 13. The skewness

and kurtosis of each mental health assessment score were 0.20-1.42 and -0.47-2.82, respectively.

3.2.6 Distributions of Serum Glucose Level

GUSTO retinal study only

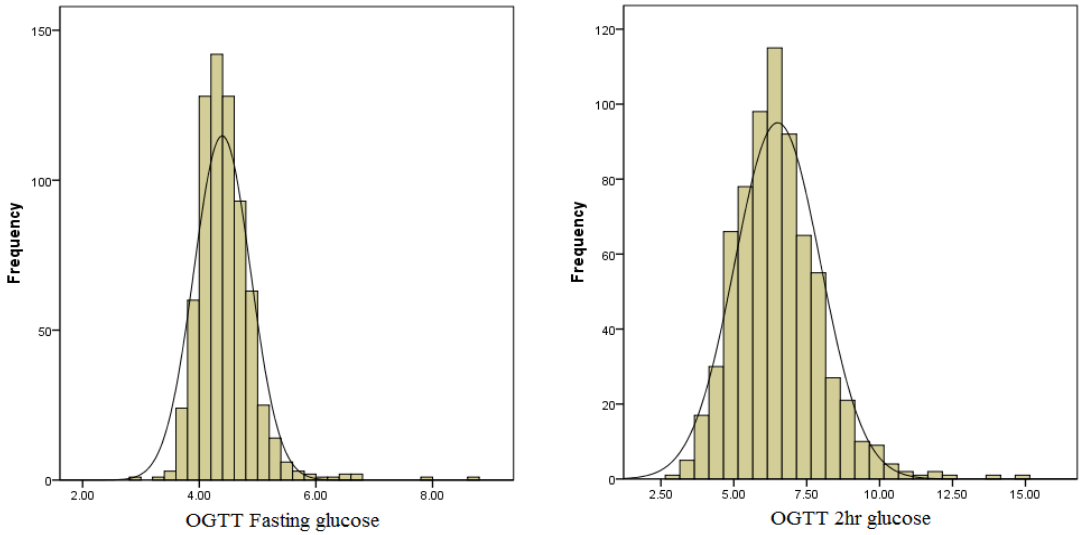


Figure 14.

The distributions of fasting glucose and 2hr-serum glucose are shown in Figure 13. The skewness and kurtosis of fasting glucose level were 2.28 and 14.01, respectively. The skewness and kurtosis of 2hr-serum glucose level were 0.95 and 2.79, respectively.

3.2.7 Distributions of Retinal Vessel Assessment

A. STARS and STARS Family retinal study (Zone B)

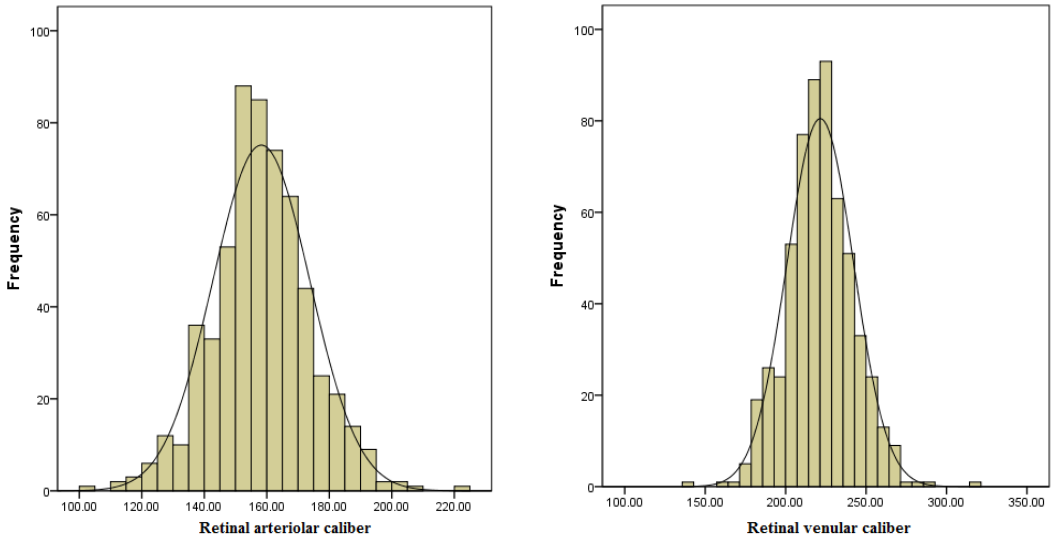
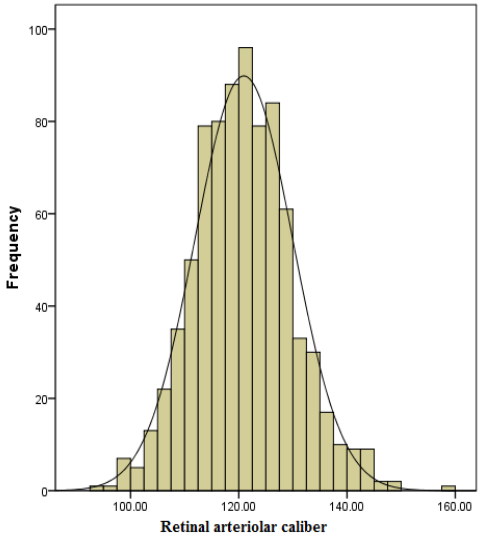
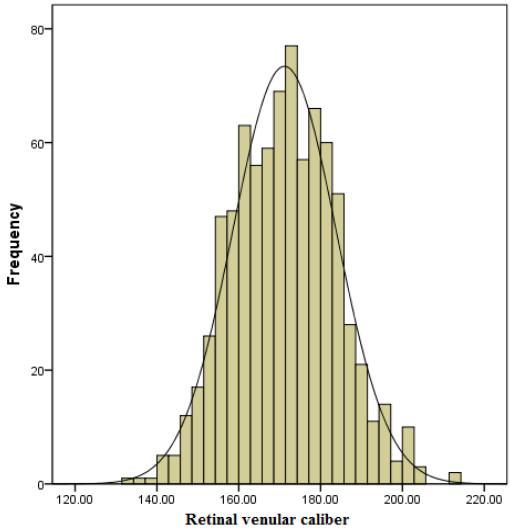


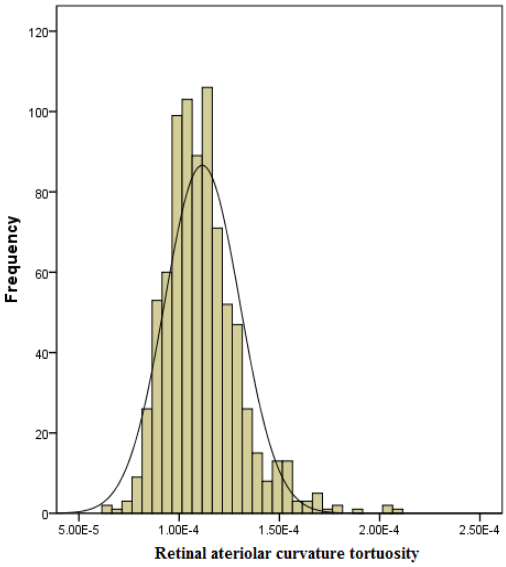
Figure 15A.

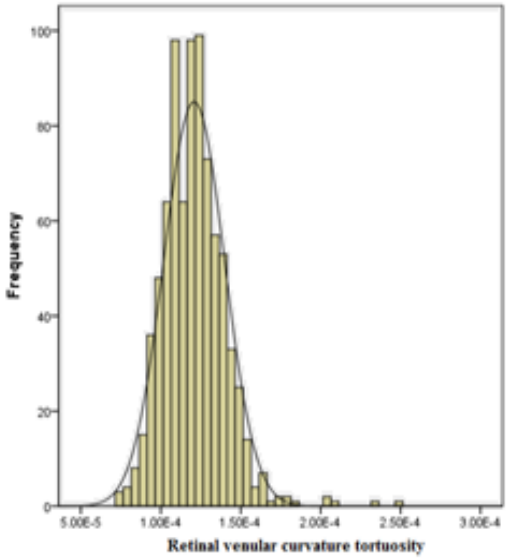
The distributions of retinal arteriolar caliber and retinal venular caliber in children were shown in **Figure 15A**. The skewness and kurtosis are 0.54 and 0.81 for retinal arteriolar caliber and 0.14 and 0.90 for retinal venular caliber, respectively. Both caliber measurements were approximately normally distributed.

B. GUSTO retinal study (Zone C)









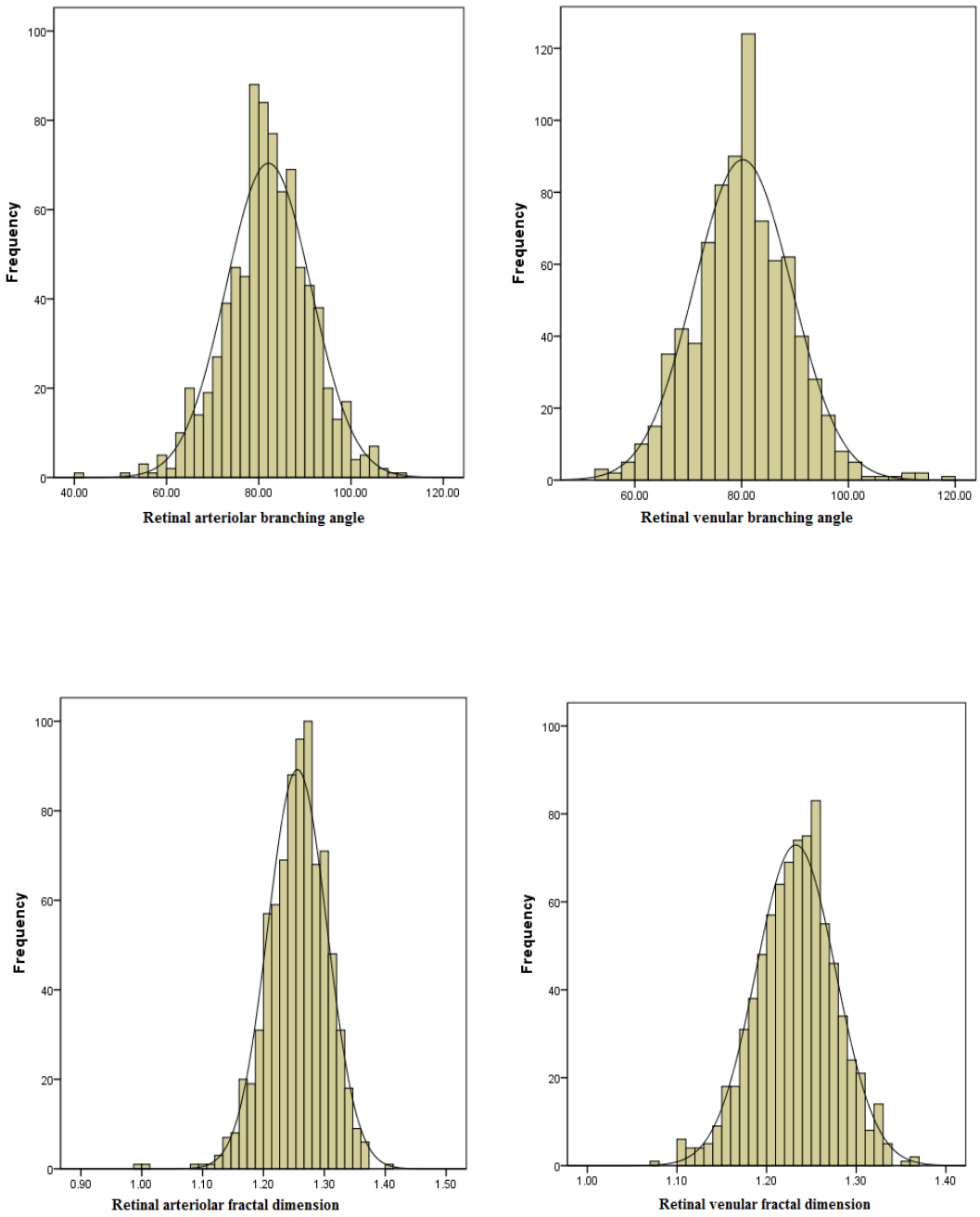


Figure 15B.

The distributions of retinal vascular caliber, tortuosity, branching angle and fractal dimension were shown in **Figure 15B**. The skewness ranged from -0.53 to 1.07 in all retinal vascular parameters and the kurtosis ranged from -0.12 to 4.58 for all retinal vascular parameters, respectively.

3.3 Prevalence of All Variables Classified by Clinical Cut-off

3.3.1 Prevalence of Hypertension

A. STARS and STARS Family retinal Study

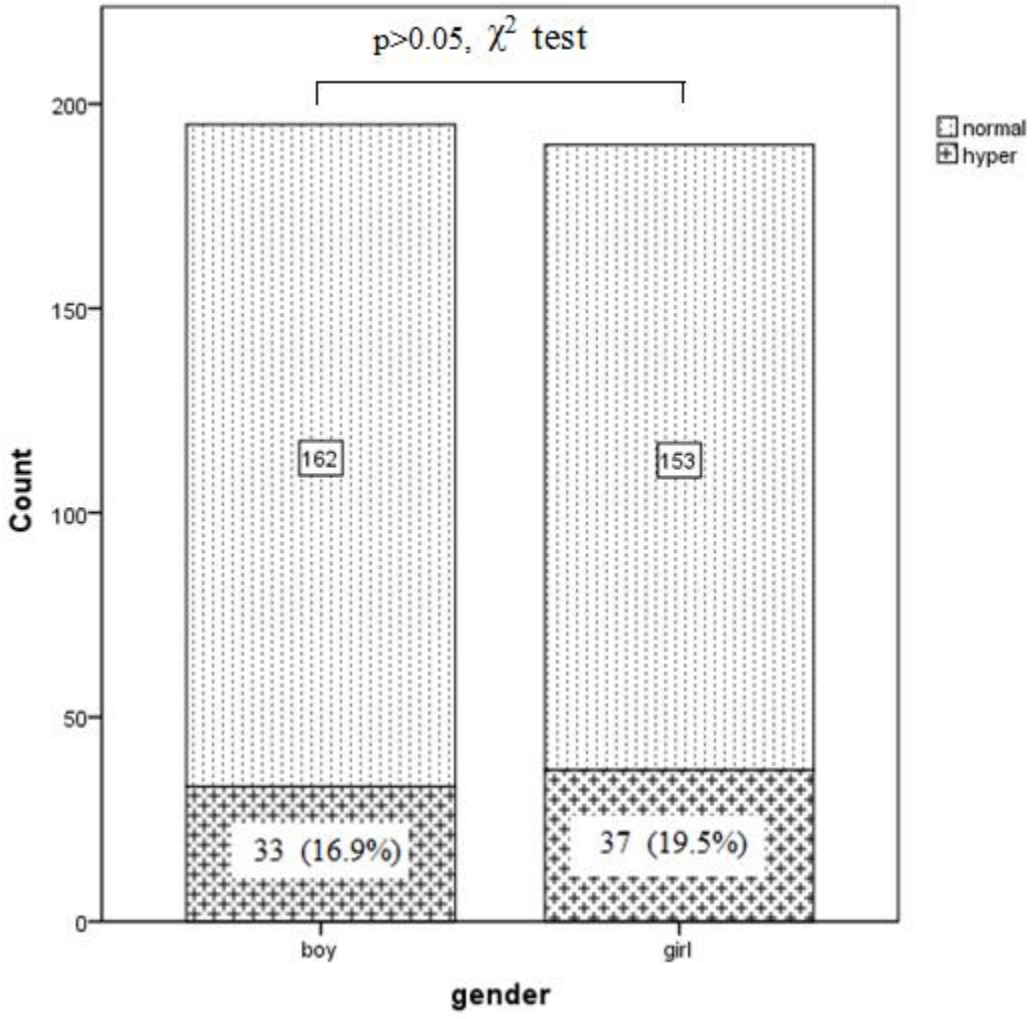


Figure 16A. Prevalence of age, gender and height-specific children hypertension

The prevalence of children with hypertension was shown in **Figure 16A.** Among 385 children aged 4-5 years, there was no difference between the prevalence of age, gender and height-adjusted hypertension between boys and girls.

B. GUSTO retinal Study

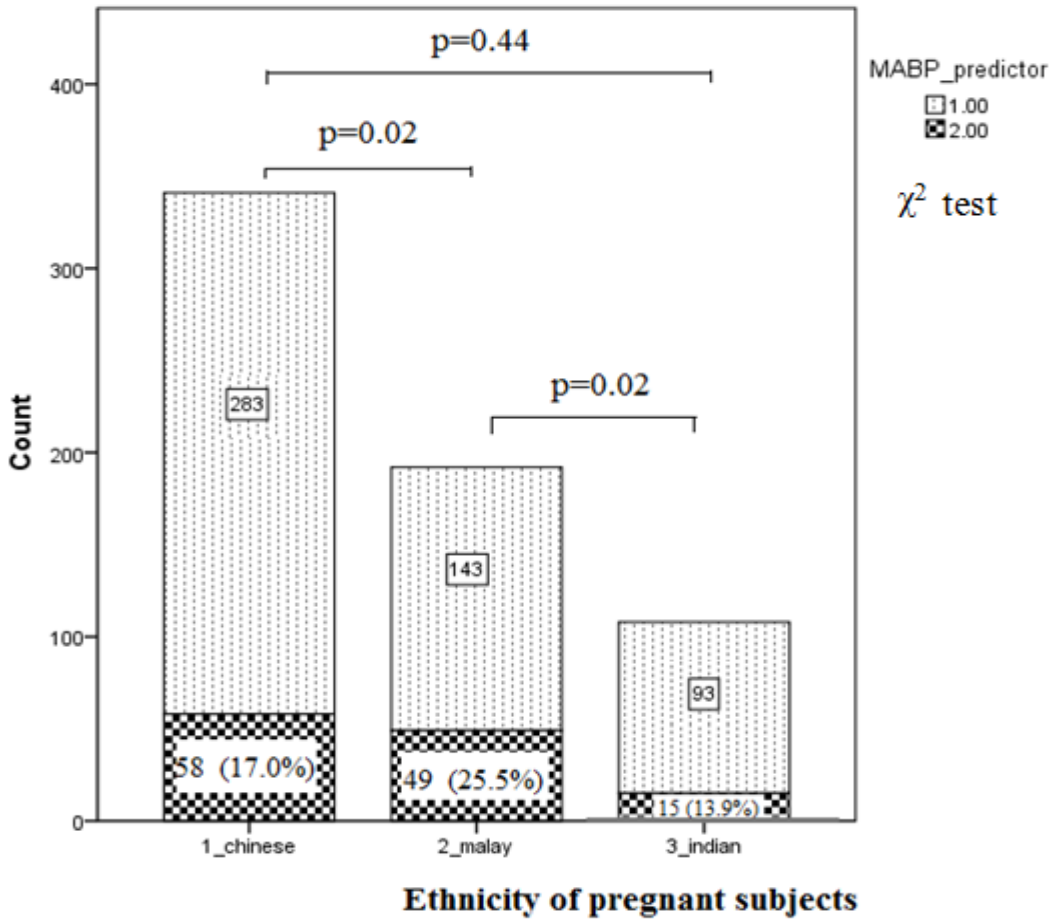


Figure 16B. Prevalence of high risk group in pre-eclampsia among Chinese, Malay and Indian pregnant subjects

By using MABP predictive value cut-off (mentioned in Methods), the prevalence of women classified into high risk group of getting future pre-eclampsia among Chinese, Malay and Indian pregnant subjects are shown in **Figure 16B**. Among 665 subjects, a total of 58, 49 and 15 Chinese, Malay and Indian pregnant women were classified accordingly into pre-eclampsia risk group. Malay subjects had a significant higher risk group than the other two ethnic groups.

3.3.2 Prevalence of Overweight and/or Obesity

A. STARS and STARS Family retinal study

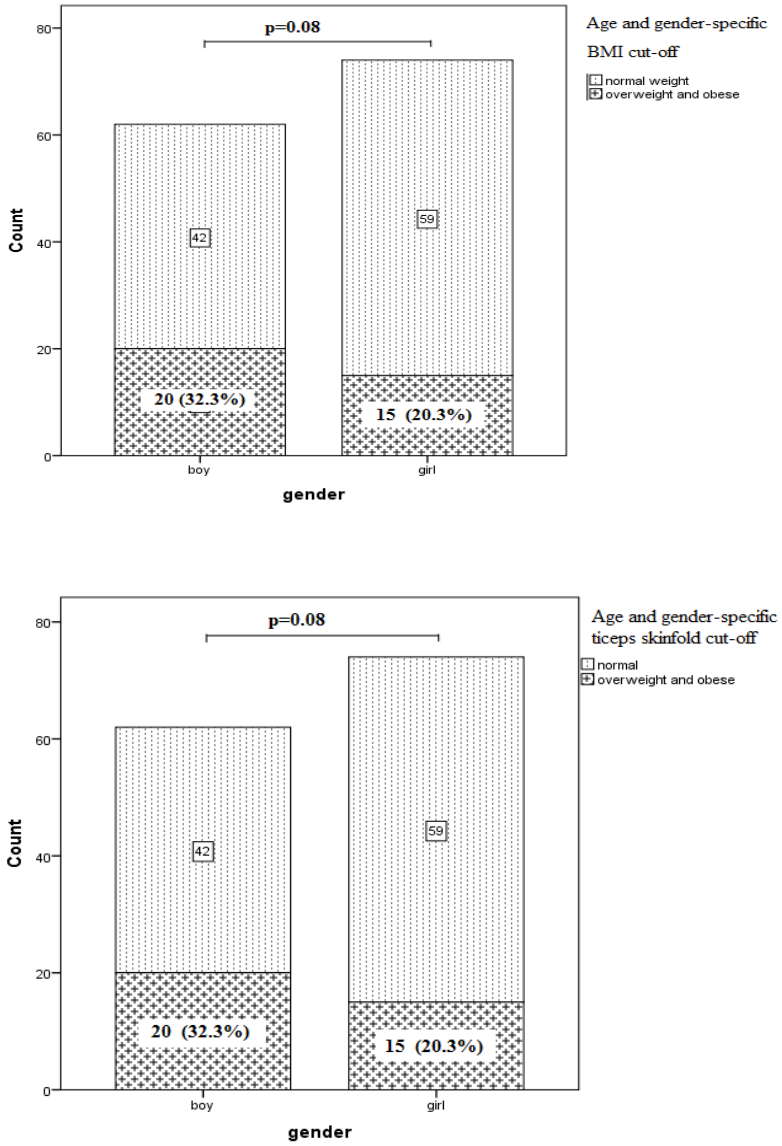


Figure 17A. Prevalence of overweight and obesity among children

By using BMI and triceps skinfold threshold, the absolute number classified into 85th percentile and above in boys and girls were the same. The prevalence of overweight and obesity in both groups (N=136) didn't vary significantly by χ^2 test.

B. GUSTO retinal study

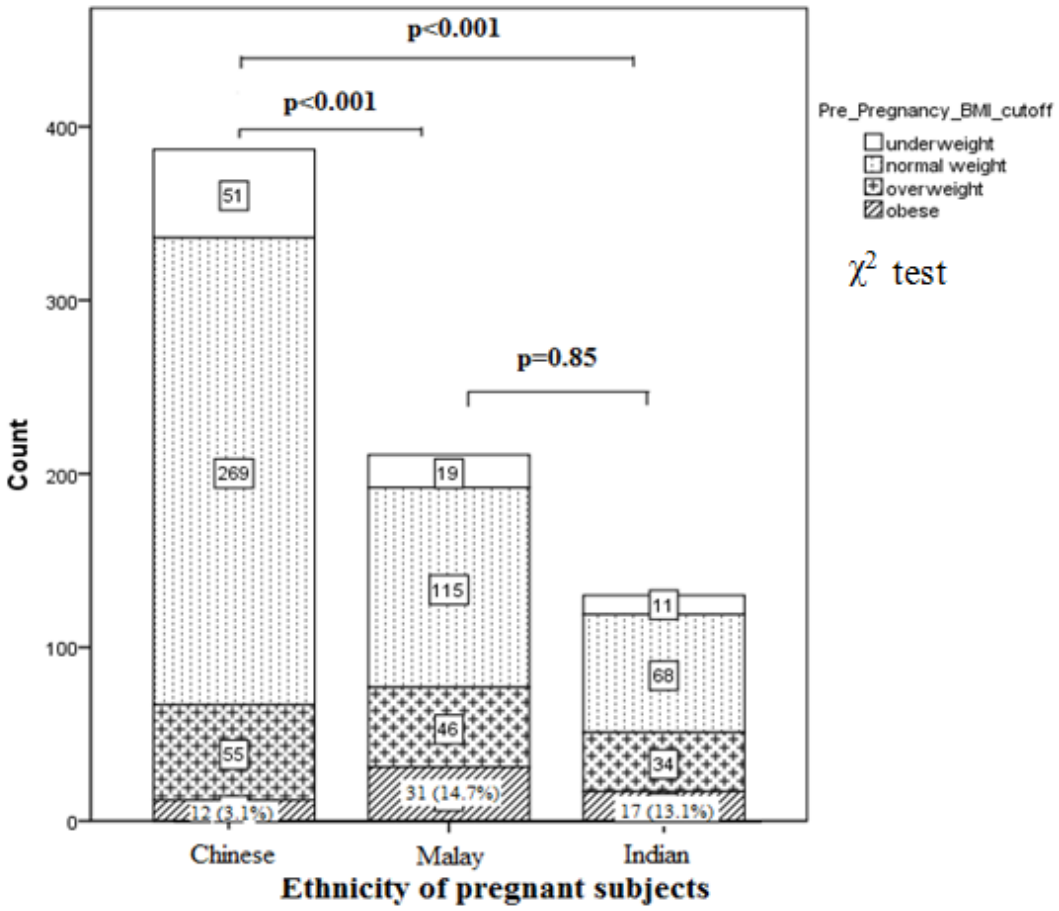


Figure 17B. Prevalence of obesity among pregnant women

By using WHO guideline, maternal obesity was defined among all ethnic groups as pre-pregnancy BMI of 30 kg/m^2 and above. Among 814 participants, Chinese had 12 obese participants, while Malay and Indian had 31 and 17 obese subjects. Using χ^2 test to compare every two groups, the prevalence of obesity among Malay and India were significantly higher than that of Chinese. The p values are shown in **Figure 17B**.

3.3.3 Prevalence of Refractive Error among Children

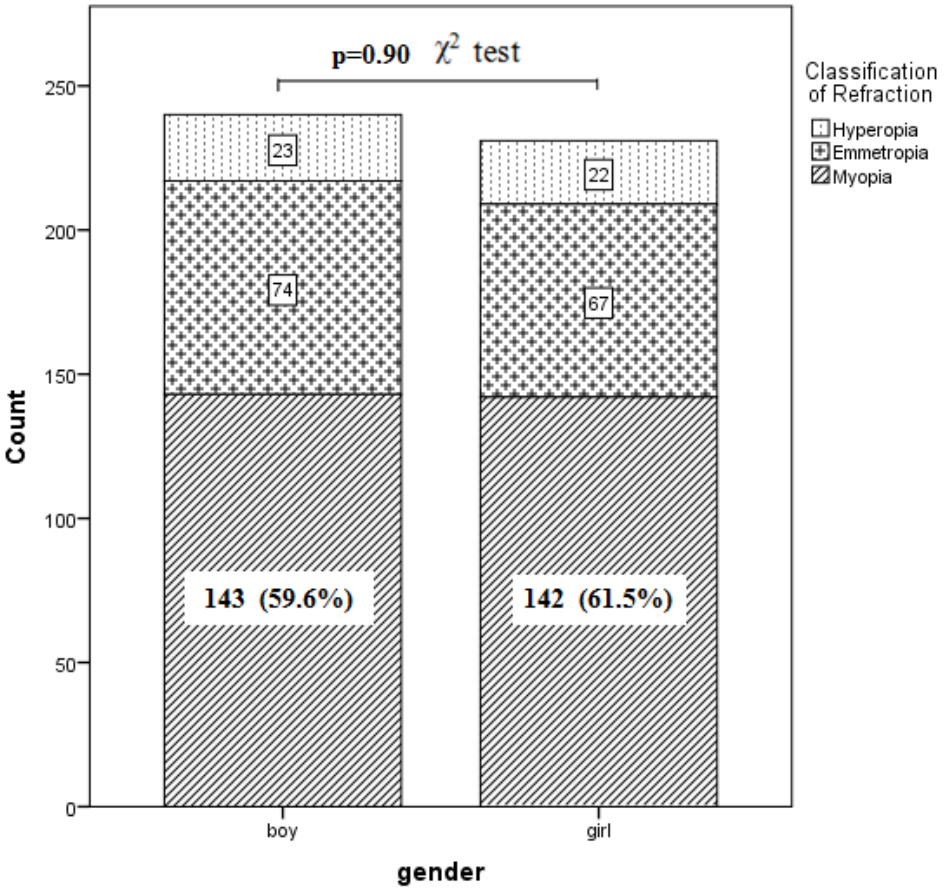


Figure 18. Prevalence of classification of refraction among children

The prevalence of classification of refraction including hyperopia, emmetropia and myopia in boys and girls was shown in **Figure 18**. In a total of 471 children aged 4-6 years, there were 143 (59.6%) myopic subjects in boys while there were 142 (61.5%) myopic subjects in girls. There was no difference in myopia prevalence between both groups (p=0.90).

3.3.4 Prevalence of Antenatal Depression among Pregnant Women

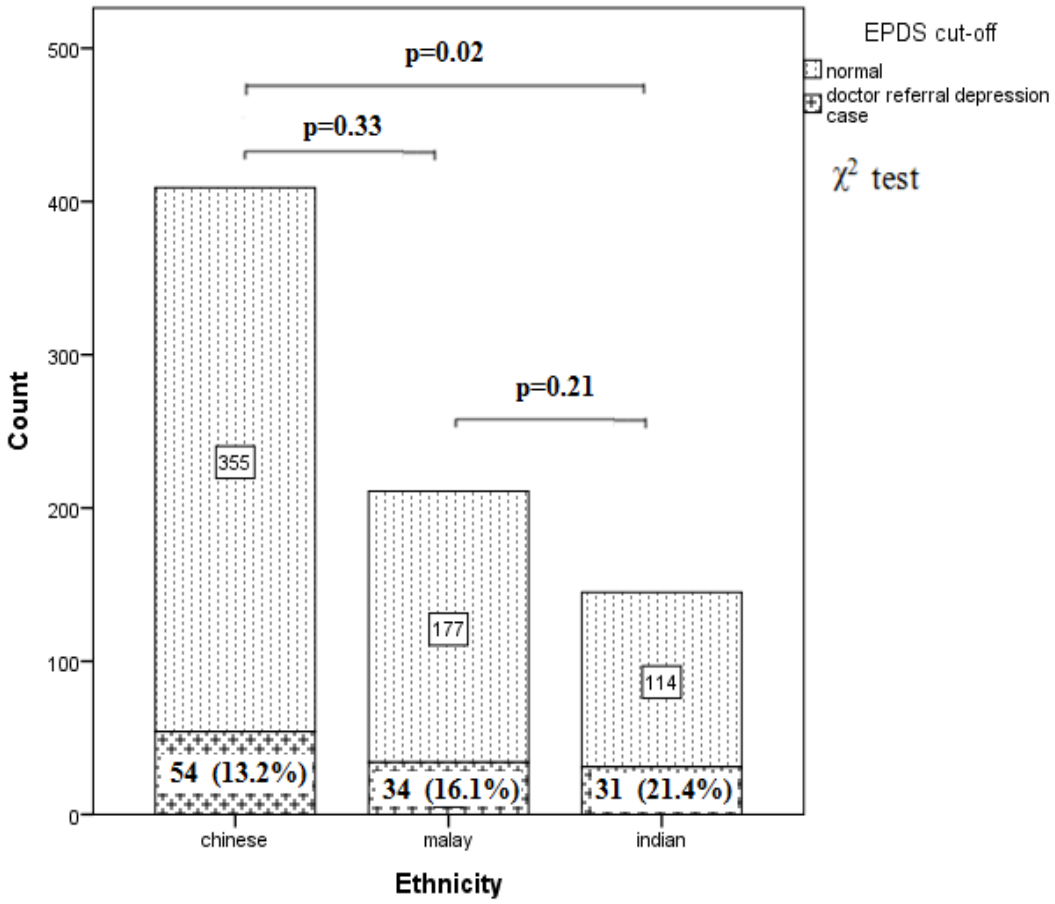


Figure 19. Prevalence of doctor-referred depression among pregnant women by using EPDS score

By using sensitive and specific depression evaluation score EPDS, a total of 119 pregnant women were defined as doctor referral depression with EPDS as 14 and above. The prevalence of such depression was the lowest in Chinese, which was also significantly different from the prevalence of antennal depression in Indian (p=0.02, **Figure 19**).

3.3.5 Prevalence of Antenatal Poor Sleep Quality among Pregnant Women

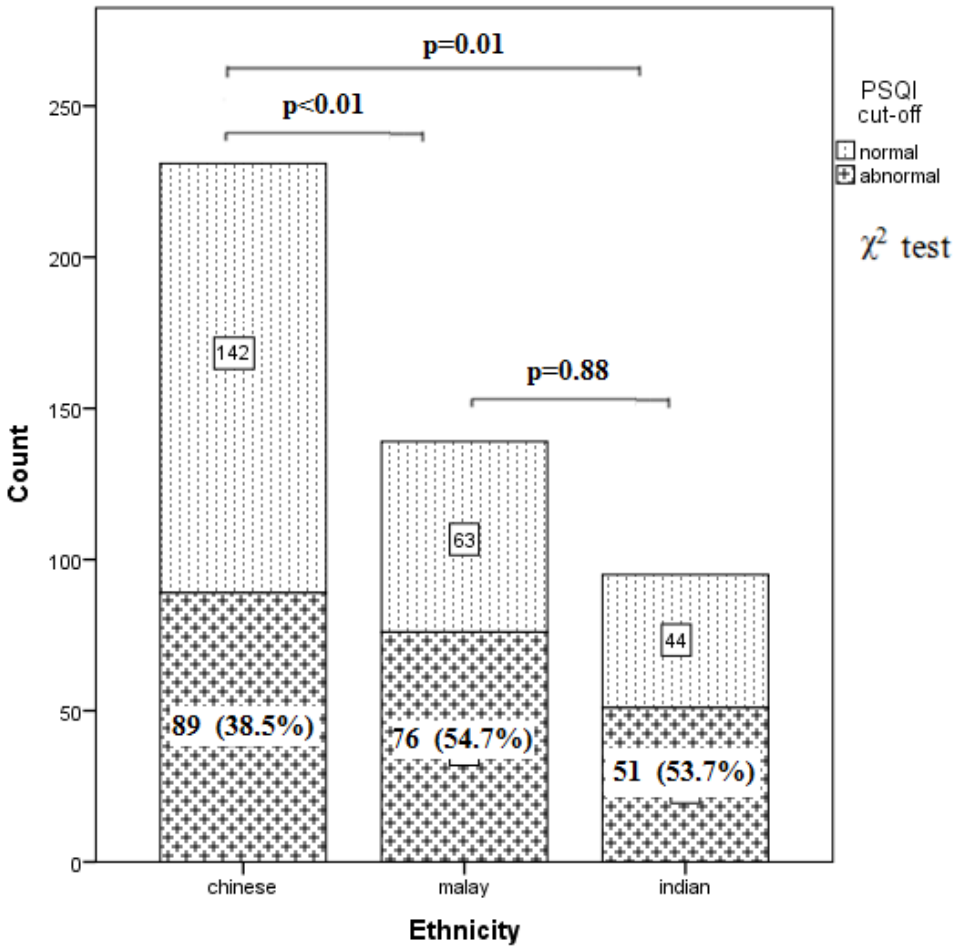


Figure 20. Prevalence of poor sleep quality among pregnant women

The prevalence of poor sleep quality among three ethnic groups was shown in **Figure 20**. There were a total of 89 cases in Chinese, 76 cases in Malay and 51 cases in Indian, respectively. Chinese pregnant participants had the lowest rate of poor sleep quality compared with Malays ($p<0.01$) and Indians ($p=0.01$).

3.3.6 Prevalence of Weight Gain Classification among Pregnant Women

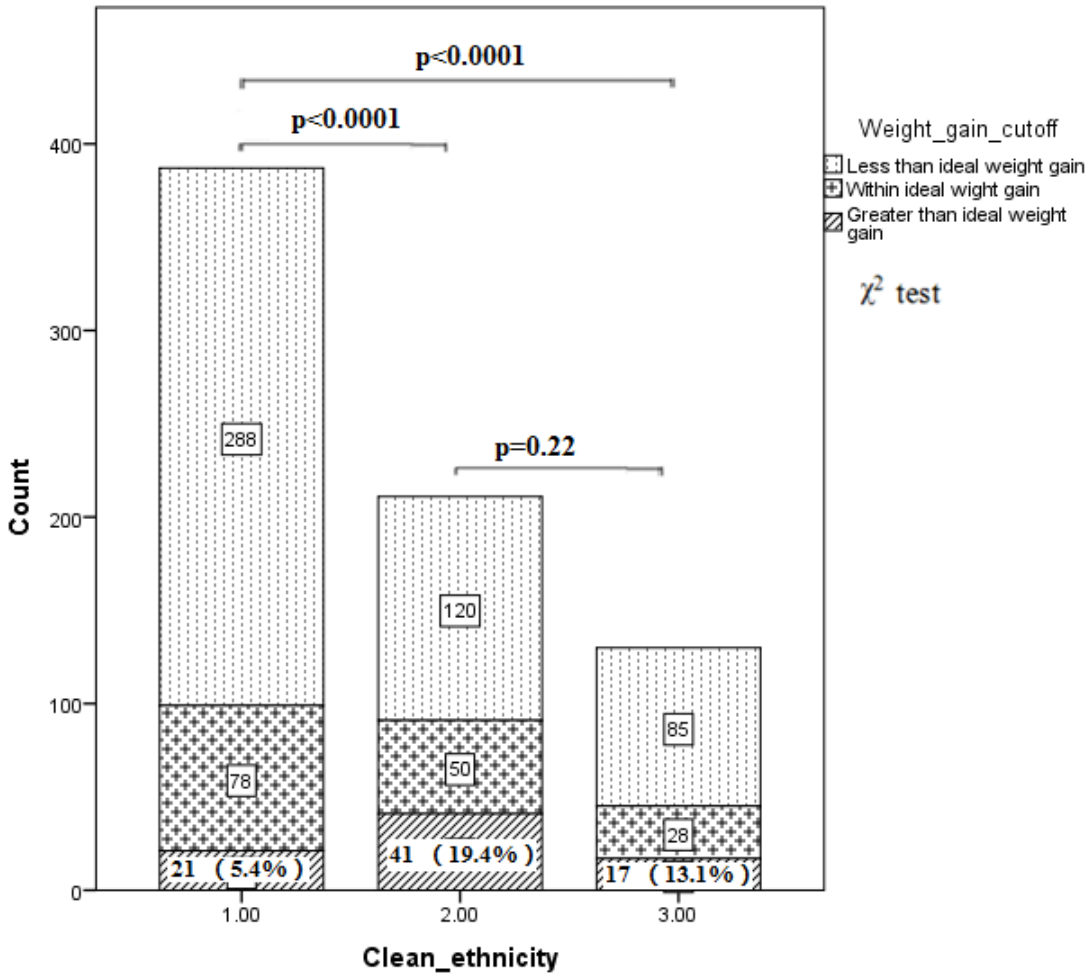


Figure 21. Prevalence of weight gain cut-off among pregnant women

The prevalence of weight gain cut-off according to WHO guideline was shown in **Figure 21**. Chinese pregnant women whose weight gain at 26 weeks were classified into greater than ideal weight gain had significantly fewer cases than Malay and Indian pregnant women ($p<0.0001$).

3.4 Risk Factors for Retinal Vascular Parameters Changes

3.4.1 Univariate Analysis

A. STARS and STARS Family retinal study

Table 28. Risk Factors (categorical variables) for Retinal Vascular Caliber Changes in Children

		CRAE		CRVE	
Categorical Variables	**N**	**mean (SD)**	**p trend**	**mean (SD)**	**p trend**
Gender					
Boys	289	155.34 (0.90)	<0.001	218.80 (19.68)	0.002
Girls	297	161.07 (0.89)		224.17 (21.43)	
Demographic status					
Father's education					
≤Secondary school,	135	157.99 (14.90)	0.42	220.40 (20.75)	0.55
O/N level,	85	158.65 (14.94)		222.36 (20.13)	
A levels/diploma,	107	160.43 (17.79)		224.73 (20.57)	
University education,	127	159.09 (15.41)		221.22 (19.97)	
Parental life style					
Mother smoking					
Current or past smoker	32	158.15 (13.21)	0.39	222.71 (21.01)	0.86
Non-smoker	554	159.3 (15.80)		221.94 (20.34)	
Father smoking					
Current or past smoker	174	158.38 (16.01)	0.23	223.80 (19.52)	0.44
Non-smoker	412	160.80 (12.82)		220.96 (20.08)	
Mother smoking during pregnancy					
Yes	8	159.78 (6.77)	0.34	223.98 (13.59)	0.70
No	578	159.04 (15.72)		221.95 (20.40)	
Mother alcohol drinking during pregnancy					
Yes	3	158.37 (0.86)	0.94	220.01 (16.79)	0.87
No	449	159.05 (15.68)		222.00 (20.33)	

Table 29. Risk Factors (continuous variables) for Retinal Vascular Caliber Changes in Children

Variables		CRAE		CRVE	
(each unit increase)	**N**	**β (SE)**	**p**	**β (SE)**	**p**
Age, years	586	-0.67 (2.23)	<0.01	-0.15 (0.31)	0.61
Blood pressure measurements					
SBP, mm Hg	514	-0.20 (0.05)	<0.001	-0.01 (0.07)	0.92
DBP, mm Hg	514	-0.15 (0.08)	0.06	0.09 (0.11)	0.43
MABP, mm Hg	514	-0.22 (0.08)	<0.01	0.05 (0.10)	0.65
Anthropometric measurements					
Weight, kg	586	-0.20 (0.06)	0.001	0.01 (0.08)	0.90
Height, cm	586	-0.13 (0.04)	<0.001	-0.304 (0.05)	0.48
BMI, kg/m^2	586	-0.14 (0.23)	0.04	0.25 (0.31)	0.42
TSF, mm	525	-0.32 (0.19)	0.09	0.34 (0.25)	0.17
Ocular biometric parameters					
Axial length, mm	454	-3.72 (0.96)	<0.001	-5.29 (1.23)	<0.001
Anterior chamber depth, mm	430	-1.27 (3.18)	0.69	-6.89 (4.13)	0.10
Corneal curvature, mm	411	-6.23 (3.24)	0.06	-10.26 (4.20)	0.02
Refraction					
Spherical equivalent, D	586	0.92 (0.30)	<0.01	1.13 (0.40)	<0.01
Birth parameters					
Birth weight, kg	435	-0.02 (1.61)	0.99	-2.01 (2.09)	0.34
Birth length, cm	431	-0.05 (0.33)	0.88	-0.24 (0.43)	0.57
Head circumference, cm	430	0.55 (0.48)	0.25	0.90 (0.63)	0.16
Gestational age, wks	89	-0.56 (0.81)	0.49	0.06 (1.28)	0.05

Table 28 and **Table 29** show the association between major variables and retinal vascular caliber among children 4-16 years by using univariate analysis. Age, gender, blood pressure, anthropometric parameters, ocular biometric indices, refractive error were potential risk factors for retinal vascular caliber changes in children and were further studied in multivariate analysis.

In univariate analysis, girls tended to have wider retinal arterioles (161.07 vs. 155.34 µm; $p<0.001$) and wider retinal venules (224.17 vs. 218.80 µm; $p=0.002$) than boys. Each one year increase in children was associated with a 0.67 µm narrowing in retinal arteriolar caliber ($p<0.01$). Each 1 mm Hg increase in SBP was associated with a 0.20 µm narrowing in retinal arteriolar caliber ($p<0.001$). Each 1.0 kg/m^2 increase in BMI was associated with a 0.14 µm narrowing in retinal arteriolar caliber ($p=0.04$). For Ocular biometrics, each 1 mm increase in axial length was associated with a 3.72 µm narrowing in retinal arteriolar caliber ($p<0.001$) and a 5.29 narrowing in retinal venular caliber ($p<0.001$), respectively. Each 1 mm increase in corneal curvature was associated with a 10.26 µm narrowing in retinal venular caliber ($p=0.02$). Each one diopter increase in spherical equivalent was associated with a 0.92 µm ($p<0.01$) widening in retinal arteriolar caliber and a 1.13 µm ($p<0.01$) widening in venular caliber, respectively.

B. GUSTO retinal study

Table 30. Risk Factors (categorical variables) for Retinal Vascular Caliber Changes in Pregnant Women

		CRAE		CRVE	
Categorical Variables	**N**	**mean (SD)**	**p trend**	**mean (SD)**	**p trend**
Ethnicity					
Chinese	450	119.88 (8.95)	0.02	169.45 (12.11)	0.22
Malay	235	121.95 (9.12)		174.84 (12.84)	
India	139	121.97 (8.99)		170.84 (12.15)	
In vitro Fertilization (IVF)					
Yes	67	119.96 (9.17)	0.35	168.27 (12.28)	0.05
No	757	121.02 (9.02)		171.50 (12.33)	
Primiparious status					
Yes	420	121.54 (8.59)	0.10	172.66 (12.51)	<0.01
No	404	120.48 (9.38)		169.86 (12.50)	
Household income, SGD/month					
1. 0-1999	162	121.55 (8.61)	0.25	172.70 (13.80)	0.13
2. 2000-3999	280	120.44 (9.09)		171.69 (12.38)	
3. 4000-5999	209	122.07 (9.58)		171.16 (12.78)	
4. >=6000	173	119.75 (8.43)		168.29 (11.33)	
Hypertension history					
Yes	19	113.88 (10.69)	0.001	165.19 (16.89)	0.04
No	805	121.07 (8.90)		171.36 (12.51)	
Diabetes history					
Yes	8	121.92 (6.64)	0.75	170.17 (16.55)	0.82
No	816	120.88 (9.03)		171.22 (12.62)	
Cigarette smoking history					
Yes	122	122.04 (9.09)	0.01	171.54 (11.33)	<0.001
No	702	120.58 (8.87)		170.60 (12.59)	
Alcohol consumption history					
Yes	255	120.96 (9.09)	0.844	170.60 (12.59)	0.33
No	569	120.82 (8.70)		171.54 (12.40)	

Table 31. Risk Factors (categorical variables) for Retinal Vascular Tortuosity Changes in Pregnant Women

Categorical Variables	N	Tortuosity A. ($x10^{-6}$) mean (SD)	p trend	Tortuosity V. ($x10^{-6}$) mean (SD)	p trend
Ethnicity					
Chinese	450	1.09 (0.19)	0.001	1.18 (0.18)	0.001
Malay	235	1.10 (0.18)		1.21 (0.18)	
India	139	1.20 (0.21)		1.28 (0.24)	
In vitro Fertilization (IVF)					
Yes	67	1.05 (0.19)	0.08	1.17 (0.19)	0.001
No	757	1.12 (0.19)		1.21 (0.19)	
Primiparious status					
Yes	420	0.11 (0.20)	0.70	0.12 (0.18)	0.57
No	404	0.11 (0.18)		0.12 (0.20)	
Household income, SGD/month					
1. 0-1999	162	1.11 (0.19)	0.98	1.24 (0.24)	0.05
2. 2000-3999	280	1.10 (0.17)		1.22 (0.22)	
3. 4000-5999	209	1.12 (1.19)		1.21 0.17)	
4. >=6000	173	1.10 (1.20)		1.18 (0.17)	
Hypertension history					
Yes	19	0.10 (0.11)	0.07	0.12 (0.17)	0.37
No	805	0.11 (0.19)		0.12 (0.19)	
Diabetes history					
Yes	8	1.15 (0.27)	0.60	1.21 (0.23)	0.96
No	816	1.11 (0.18)		1.21 (0.19)	
Cigarette smoking history					
Yes	122	1.13 (0.18)	0.28	1.23 (0.20)	0.30
No	702	1.11 (0.19)		1.21 (0.19)	
Alcohol consumption history					
Yes	255	1.12 (0.20)	0.56	1.21 (0.20)	0.68
No	569	1.11 (0.19)		1.21 (0.19)	

Table 32. Risk Factors (categorical variables) for Retinal Vascular Branching Angle Changes in Pregnant Women

Categorical Variables	N	Branching angle A. mean (SD)	p trend	Branching angle V. mean (SD)	p trend
Ethnicity					
Chinese	450	81.22 (9.46)	0.001	79.89 (9.05)	<0.01
Malay	235	82.01 (9.59)		79.60 (8.82)	
India	139	84.22 (8.22)		82.55 (9.54)	
In vitro Fertilization (IVF)					
Yes	67	80.31 (8.56)	0.13	82.01 (8.43)	0.10
No	757	82.13 (9.36)		80.05 (9.18)	
Primiparious status					
Yes	420	81.56 (8.85)	0.20	79.75 (9.16)	0.13
No	404	82.43 (9.80)		80.75 (9.06)	
Household income, SGD/month					
1. 0-1999	162	80.88 (4.70)	0.24	78.99 (8.93)	0.59
2. 2000-3999	280	82.25 (8.90)		81.42 (8.87)	
3. 4000-5999	209	82.11 (10.10)		79.73 (9.16)	
4. >=6000	173	82.40 (9.63)		79.36 (9.46)	
Hypertension history					
Yes	19	77.41 (9.95)	0.03	80.16 (10.43)	0.96
No	805	82.21 (9.23)		80.28 (9.14)	
Diabetes history					
Yes	8	82.73 (15.92)	0.85	87.05 (5.64)	0.04
No	816	82.09 (9.20)		80.21 (9.17)	
Cigarette smoking history					
Yes	122	82.43 (8.30)	0.55	79.82 (9.75)	0.57
No	702	81.88 (9.50)		80.33 (8.98)	
Alcohol consumption history					
Yes	255	81.24 (9.36)	0.15	79.93 (8.96)	0.51
No	569	82.28 (9.31)		80.39 (9.16)	

Table 33. Risk Factors (categorical variables) for Retinal Vascular Fractal Dimension Changes in Pregnant Women

Categorical Variables	N	Fractal dimension A. ($x10^{-3}$) mean (SD)	p trend	Fractal dimension V. ($x10^{-3}$) mean (SD)	p trend
Ethnicity					
Chinese	450	1.25 (0.05)	<0.01	1.23 (0.05)	0.49
Malay	235	1.26 (0.05)		1.24 (0.04)	
India	139	1.26 (0.04)		1.23 (0.04)	
In vitro Fertilization (IVF)					
Yes	67	1.26 (0.04)	0.99	1.23 (0.05)	0.91
No	757	1.26 (0.05)		1.23 (0.04)	
Primiparious status					
Yes	420	1.26 (0.05)	0.49	1.23 (0.05)	0.47
No	404	1.26 (0.05)		1.23 (0.04)	
Household income, SGD/month					
1. 0-1999	162	1.26 (0.05)	0.04	1.24 (0.04)	0.01
2. 2000-3999	280	1.25 (0.05)		1.24 (0.05)	
3. 4000-5999	209	1.26 (0.05)		1.23 (0.04)	
4. >=6000	173	1.25 (0.05)		1.22 (0.04)	
Hypertension history					
Yes	19	1.23 (0.06)	0.01	1.23 (0.05)	0.48
No	805	1.26 (0.05)		1.23 (0.04)	
Diabetes history					
Yes	8	1.24 (0.05)	0.24	1.22 (0.27)	0.59
No	816	1.26 (0.05)		1.23 (0.04)	
Cigarette smoking history					
Yes	122	1.26 (0.05)	0.66	1.24 (0.05)	0.12
No	702	1.26 (0.05)		1.23 (0.04)	
Alcohol consumption history					
Yes	255	1.25 (0.05)	0.34	1.23 (0.04)	0.95
No	569	1.26 (0.05)		1.23 (0.05)	

Table 30-33 shows associations between major categorical variables of interest and a set of retinal vascular parameters (caliber, curvature tortuosity, branching angle and fractal dimension) by using univariate analysis. Ethnicity, conception of IVF, primiparious status of current pregnancy, household income, hypertension history and cigarette smoking history were all associated with retinal vascular characteristics changes. For example, compared with Malays and Indians, Chinese subjects had the narrowest retinal arterioles (119.88 vs. 121.95 vs. 121.97 μm, p=0.02), the least tortuous retinal arterioles (1.09 vs. 1.10 vs. 1.20, p-0.001) and retinal venules (1.18 vs. 1.21 vs. 1.28, p=0.001), the smallest branching angle in retinal arterioles (81.22 vs. 82.01 vs. 84.22, p=0.001) and the smallest retinal arteriolar fractal dimension (1.25 vs. 1.26 vs. 1.26, p<0.01).

Among all pregnant women, Chinese tended to have the lowest retinal arteriolar and venular parameters. Conception through In-vitro Fertilization (IVF) tended to be associated with less retinal venular tortuosity and narrower retinal venular caliber. Participants in lower household income (SGD 0-1999/month) also had wider retinal venular caliber, less retinal tortuous venules and smaller retinal venular fractal dimension, compared with higher household income (SGD 6000 and above/month). Pregnant women who had hypertension history had narrower retinal arteriolar caliber, less retinal arteriolar tortuosity, smaller retinal arteriolar branching angle and smaller retinal arteriolar fractal dimension accordingly. Participants who had chronic hypertension tended to have narrower retinal arteriolar caliber (113.88 vs. 121.07 μm, p=0.001), smaller retinal arteriolar branching angle (77.41 vs. 82.21, p=0.03) and smaller retinal arteriolar fractal dimension (1.23 vs. 1.26, p=0.01), compared with

patients without hypertension history. Furthermore, cigarette smoking tended to have an impact on retinal vascular network, such as retinal arteriolar narrowing and retinal venular widening. Pregnant women who had current or past cigarette smoking history tended to have wider retinal venular caliber (171.54 vs. 171.22 μm, $p<0.001$) than non-smokers.

Therefore, these variables of interest were further analyzed in the multivariate regression models.

Table 34. Risk Factors (continuous variables) for Retinal Vascular Caliber Changes in Pregnant Women

Variables		CRAE		CRVE	
(each unit increase)	**N**	**β (SE)**	**p**	**β (SE)**	**p**
Age, years	824	-0.13 (0.06)	0.03	-0.40 (0.08)	<0.001
Blood pressure measurements					
SBP, mm Hg	665	-0.14 (0.03)	<0.001	-0.08 (0.04)	0.05
DBP, mm Hg	665	-0.23 (0.04)	<0.001	-0.08 (0.05)	0.11
MABP, mm Hg	665	-0.24 (0.04)	<0.001	-0.11 (0.05)	0.05
Anthropometric measurements					
Height, cm	824	0.01(0.04)	0.850	-0.02 (0.06)	0.66
Pre-pregnancy weight, kg	741	-0.09 (0.03)	0.001	-0.03 (0.04)	0.48
Pregnancy weight, kg	814	-0.10 (0.02)	<0.001	-0.02 (0.03)	0.65
Weight gain at 26 wks, kg	741	-0.03 (0.07)	0.70	0.09 (0.10)	0.33
Pre-pregnancy BMI, kg/m^2	741	-0.26 (0.07)	<0.001	-0.03 (0.10)	0.78
Pregnancy BMI, kg/m^2	814	-0.29 (0.07)	<0.001	0.00 (0.09)	0.99
MUAC, mm	813	-0.31 (0.07)	<0.001	0.01 (0.10)	0.95
Triceps skinfold, mm	813	-0.21 (0.05)	<0.001	-0.08 (0.07)	0.27
Biceps skinfold, mm	814	-0.15 (0.06)	0.011	0.00 (0.08)	0.96
Subscalpular skinfold, mm	813	-0.20 (0.05)	<0.001	-0.09 (0.07)	0.18
Suprailiac skinfold, mm	812	-0.14 (0.06)	0.011	-0.02 (0.08)	0.77
Refraction					
Spherical equivalent, D	824	1.17 (0.11)	<0.001	1.49 (0.16)	<0.001
Mental health assessments					
EPDS, points	824	0.17 (0.07)	0.02	0.22 (0.10)	0.03
BDI-II, points	817	0.08 (0.05)	0.08	0.20 (0.07)	<0.01
STAI, points	801	0.02 (0.02)	0.29	0.03 (0.03)	0.17
PSQI, points	483	0.50 (0.15)	0.001	0.54 (0.20)	<0.01

Table 35. Risk Factors (continuous variables) for Retinal Vascular Tortuosity Changes in Pregnant Women

Variables		Tortuosity A. ($x10^{-6}$)		Tortuosity V. ($x10^{-6}$)	
(each unit increase)	**N**	**β (SE)**	**p**	**β (SE)**	**p**
Age, years	824	-0.01 (0.001)	0.001	-0.004 (0.001)	<0.01
Blood pressure measurements					
SBP, mm Hg	665	-0.04 (0.06)	0.54	0.03 (0.06)	0.58
DBP, mm Hg	665	-0.11 (0.08)	0.14	0.08 (0.08)	0.31
MABP, mm Hg	665	-0.10 (0.08)	0.20	0.08 (0.08)	0.34
Anthropometric measurements					
Height, cm	824	0.18 (0.13)	0.44	0.17 (0.13)	0.21
Pre-pregnancy weight, kg	741	0.01 (0.06)	0.93	0.20 (0.06)	0.001
Pregnancy weight, kg	814	0.08 (0.14)	0.79	0.24 (0.19)	0.001
Weight gain at 26 wks, kg	741	-0.03 (0.14)	0.83	-0.09 (0.15)	0.56
Pre-pregnancy BMI, kg/m^2	741	-0.03 (0.15)	0.85	0.53 (0.15)	0.001
Pregnancy BMI, kg/m^2	814	-0.19 (0.18)	0.53	0.57 (0.17)	0.001
MUAC, mm	813	0.08 (0.27)	0.77	0.53 (0.23)	0.001
Triceps skinfold, mm	813	0.02 (0.12)	0.66	0.23 (0.17)	0.07
Biceps skinfold, mm	814	-0.18 (0.15)	0.45	0.17 (0.14)	0.44
Subscalpular skinfold, mm	813	0.09 (0.14)	0.45	0.28 (0.13)	0.04
Suprailiac skinfold, mm	812	-0.18 (0.16)	0.57	0.21 (0.11)	0.11
Refraction					
Spherical equivalent, D	824	0.91 (0.32)	0.001	0.53 (0.38)	0.08
Mental health assessments					
EPDS, points	824	0.27 (0.16)	0.25	0.38 (0.24)	0.05
BDI-II, points	817	0.19 (0.13)	0.58	0.02 (0.01)	0.67
STAI, points	801	0.03 (0.01)	0.60	0.04 (0.05)	0.60
PSQI, points	483	0.03 (0.32)	0.88	-0.23 (0.39)	0.55

Table 36. Risk Factors (continuous variables) for Retinal Vascular Branching Angle Changes in Pregnant Women

Variables		Branching angle A.		Branching angle V.	
(each unit increase)	**N**	**β (SE)**	**p**	**β (SE)**	**p**
Age, years	824	-0.07 (0.06)	0.27	0.03 (0.06)	0.65
Blood pressure measurements					
SBP, mm Hg	665	-0.06 (0.03)	0.05	0.06 (0.03)	0.06
DBP, mm Hg	665	-0.07 (0.04)	0.07	0.10 (0.04)	0.01
MABP, mm Hg	665	-0.09 (0.04)	0.03	0.10 (0.04)	0.01
Anthropometric measurements					
Height, cm	824	0.01 (0.04)	0.98	0.02 (0.04)	0.64
Pre-pregnancy weight, kg	741	0.02 (0.03)	0.94	0.70 (0.03)	0.01
Pregnancy weight, kg	814	-0.03 (0.03)	0.90	0.08 (0.03)	<0.01
Weight gain at 26 wks, kg	741	-0.07 (0.07)	0.32	-0.01 (0.07)	0.86
Pre-pregnancy BMI, kg/m^2	741	-0.01 (0.07)	0.95	0.23 (0.07)	<0.01
Pregnancy BMI, kg/m^2	814	0.10 (0.07)	0.90	0.22 (0.07)	0.001
MUAC, mm	813	-0.03 (0.08)	0.97	0.26 (0.08)	0.001
Triceps skinfold, mm	813	-0.20 (0.05)	0.72	0.09 (0.05)	0.09
Biceps skinfold, mm	814	-0.12 (0.06)	0.05	0.14 (0.06)	0.02
Subscalpular skinfold, mm	813	-0.02 (0.05)	0.97	0.06 (0.05)	0.20
Suprailiac skinfold, mm	812	0.05 (0.06)	0.36	0.10 (0.06)	0.08
Refraction					
Spherical equivalent, D	824	0.26 (0.13)	0.04	0.28 (0.12)	0.02
Mental health assessments					
EPDS, points	824	0.06 (0.07)	0.43	0.01 (0.07)	0.87
BDI-II, points	817	0.07 (0.05)	0.16	0.04 (0.05)	0.44
STAI, points	801	0.00 (0.02)	0.82	0.02 (0.02)	0.41
PSQI, points	483	0.12 (0.15)	0.42	0.20 (0.14)	0.17

Table 37. Risk Factors (continuous variables) for Retinal Vascular Fractal Dimension Changes in Pregnant Women

Variables (each unit increase)	N	Fractal Dimension A. ($x10^{-3}$) β (SE)	p	Fractal Dimension V. ($x10^{-3}$) β (SE)	p
Age, years	824	-1.43 (0.32)	<0.001	-1.61 (0.29)	<0.001
Blood pressure measurements					
SBP, mm Hg	665	-0.53 (0.16)	0.001	0.05 (0.14)	0.75
DBP, mm Hg	665	-0.75 (0.20)	<0.001	0.09 (0.18)	0.62
MABP, mm Hg	665	-0.84 (0.21)	<0.001	0.02 (0.19)	0.63
Anthropometric measurements					
Height, cm	824	0.21 (0.30)	0.48	0.30 (0.27)	0.28
Pre-pregnancy weight, kg	741	0.15 (0.13)	0.25	-0.24 (0.15)	0.11
Pregnancy weight, kg	814	-0.26 (0.14)	0.06	0.19 (0.13)	0.13
Weight gain at 26 wks, kg	741	-0.29 (0.37)	0.44	-0.14 (0.33)	0.68
Pre-pregnancy BMI, kg/m^2	741	-0.65 (0.39)	0.10	0.36 (0.35)	0.31
Pregnancy BMI, kg/m^2	814	-0.85 (0.37)	0.02	0.41 (0.34)	0.23
MUAC, mm	813	-0.86 (0.41)	0.03	0.44 (0.37)	0.24
Triceps skinfold, mm	813	-0.31 (0.28)	0.27	0.27 (0.26)	0.30
Biceps skinfold, mm	814	-0.49 (0.32)	0.12	0.37 (0.29)	0.20
Subscalpular skinfold, mm	813	-0.49 (0.26)	0.06	-0.01 (0.24)	0.96
Suprailiac skinfold, mm	812	-0.26 (0.31)	0.39	0.12 (0.28)	0.68
Refraction					
Spherical equivalent, D	824	3.56 (0.64)	<0.001	2.49 (0.58)	<0.001
Mental health assessments					
EPDS, points	824	0.55 (0.38)	0.15	0.26 (0.35)	0.46
BDI-II, points	817	0.17 (0.26)	0.52	-0.01 (0.24)	0.97
STAI, points	801	0.10 (0.10)	0.29	-0.06 (0.09)	0.47
PSQI, points	483	0.23 (0.80)	0.78	-0.25 (0.68)	0.71

Table 34-37 shows associations between major continuous variables of interest and a set of retinal vascular parameters (caliber, curvature tortuosity, branching angle and fractal dimension) by using univariate analysis. All variables mentioned were all associated with retinal vascular characteristics changes.

Among all pregnant women, each 1 year increase in age was associated with a 0.13 μm narrowing in retinal arteriolar caliber ($p<0.03$) and a 0.40 μm narrowing in retinal venular caliber ($p<0.001$), respectively. Similarly, older age was associated with smaller retinal vascular tortuosity ($p<0.01$) and smaller retinal vascular fractal dimension ($p<0.001$). Each 1 mmHg increase in SBP, DBP and MABP was associated with 0.14-0.24 μm narrowing in retinal arteriolar caliber ($p<0.001$) and 0.53-0.84 decrease in retinal arteriolar fractal dimension ($p<0.01$). Anthropometric measurements including BMI and skinfold thickness were more strongly associated with retinal vascular caliber than other parameters. For example, each 1 kg/m^2 increase in pre-pregnancy BMI and mid-pregnancy BMI was associated with a 0.26 μm ($p<0.001$) and a 0.29 μm ($p<0.001$) widening in retinal arteriolar caliber, respectively. Refraction was associated with all retinal vascular parameters. Higher spherical equivalent was associated with increment in all retinal vascular parameters. Antenatal mental health assessments were only associated with retinal vascular caliber. For instance, each one score increase in EPDS was associated with a 0.17 μm ($p=0.02$) and a 0.22 μm ($p=0.03$) widening in retinal arteriolar and venular caliber, respectively.

Therefore, all these continuous variables of interest were further analyzed in the multivariate regression models.

3.4.2 Multivariate Analysis in Cross-sectional Results

3.4.2.1 Multiple Linear Regression

3.4.2.1.1 Blood Pressure as a Risk Factor for Changes in Retinal Vascular Parameters

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Table 38. Association between Blood Pressure and Retinal Vascular Caliber

	N	CRAE, µm Mean (SD)	p trend	CRVE, µm Mean (SD)	p trend
Systolic blood pressure, mm Hg					
1st quintile, ≤91.53	77	161.50 (18.26)	0.01	220.76 (21.57)	0.04
2nd quintile, 91.54-97.00	81	157.45 (16.27)		223.10 (19.60)	
3rd quintile, 97.01-103.50	76	159.88 (13.88)		220.28 (21.14)	
4th quintile, 103.51-109.00	79	159.58 (13.75)		224.39 (19.08)	
5th quintile, >109.00	72	157.07 (14.08)		223.69 (21.08)	
Diastolic blood pressure, mm Hg					
1st quintile, ≤56.00	80	161.75 (18.26)	0.22	220.78 (23.53)	0.21
2nd quintile, 56.01-60.00	78	157.45 (16.27)		223.03 (20.31)	
3rd quintile, 60.01-63.00	76	159.88 (13.88)		221.14 (17.93)	
4th quintile, 63.01-67.00	76	159.58 (13.75)		223.34 (19.41)	
5th quintile, >67.00	75	157.07 (14.08)		224.08 (20.83)	
Mean arterial blood pressure, mm Hg					
1st quintile, ≤68.67	78	160.91 (17.98)	0.06	220.46 (23.11)	0.10
2nd quintile, 68.68-72.67	79	158.38 (15.83)		220.88 (19.99)	
3rd quintile, 72.68-76.50	75	161.40 (13.98)		224.50 (17.68)	
4th quintile, 76.51-80.47	78	158.56 (15.33)		222.60 (21.07)	
5th quintile, >80.47	75	156.43 (13.30)		223.88 (20.22)	
Hypertension stage					
Normotensive	315	160.15 (15.54)	0.02	222.18 (20.25)	0.15
Hypertensive	70	156.06 (14.40)		223.90 (21.19)	

The mean of CRAE or CRVE was adjusted for age, gender, father's education, BMI, brith weight, axial length and fellow retinal vessel.

Table 39. Linear Regression Models of Retinal Vascular Caliber and Blood Pressure

	CRAE, µm		CRVE, µm	
Per 10 mm Hg increase	**β (95% CI)**	**p**	**β (95% CI)**	**p**
Systolic blood pressure, mm Hg				
Age and gender*	-1.56 (-3.11, -0.01)	0.05	0.80 (-1.30, 2.91)	0.45
Multivariable‡	-2.00 (-3.61, -0.39)	0.02	2.51 (0.35, 4.68)	0.02
Diastolic blood pressure, mm Hg				
Age and gender*	-0.81 (-2.35, -0.39)	0.30	0.67 (-1.38, 2.76)	0.65
Multivariable‡	-0.73 (-2.23, 0.78)	0.34	1.48 (-0.53, 3.48)	0.15
Mean arterial blood pressure, mm Hg				
Age and gender*	-1.26 (-2.81, 0.28)	0.11	0.83 (-1.26, 2.92)	0.44
Multivariable‡	-1.39 (-2.95, 0.17)	0.08	2.15 (0.06, 4.24)	0.04

*The regression coefficient of CRAE or CRVE was adjusted for age and gender.

‡ The regression coefficient of CRAE or CRVE was adjusted for age, gender, father's education, BMI, birth weight, axial length and fellow retinal vessel.

Table 38 shows the mean retinal arteriolar and venular caliber by quintiles of SBP, DBP, and MABP in different models. In the multivariate-adjusted model, children with higher SBP quintiles had consistently and significantly narrower retinal arteriolar caliber (p-trend=0.01). Narrower CRAE was significantly associated with higher SBP (p-trend= 0.01). However, there were no associations between CRAE and DBP (p-trend=0.22) or MABP (p-trend=0.06). CRVE was also associated with SBP in the multivariable-adjusted model (p-trend=0.04). Children classified as age-, gender- and height-specific hypertension (n=70) had approximately 4 µm narrower retinal arteriolar caliber (p trend=0.02) than children who were classified as normotensive (n=315) in the same multivariable analysis.

Table 39 shows the multivariate linear regression models constructed to determine the regression coefficient (β) for retinal vascular caliber for each 10 mm Hg increase

in blood pressure. In model 1 that adjusted for age and gender, CRAE decreased by 1.56-µm (95% Confidence Interval: 0.01 to 3.11-µm; p=0.05) for each10 mm Hg increase in SBP. In model 2 that adjusted for age, gender, father's education, body mass index, birth weight, axial length and fellow retinal vessel, for each 10 mm Hg increase in SBP, CRAE decreases by 2.00-µm (95% Confidence Interval: 0.39 to 3.61-µm; p=0.01) and CRVE increased by 2.51-µm (95% Confidence Interval: 0.35 to 4.68-µm; p=0.02). Similarly, each 10 mm Hg increase in MABP was associated with 2.15-µm (95% Confidence Interval: 0.06 to 4.24-µm; p=0.04) wider CRVE, but no change in CRAE. No significant association was found between a 10 mm Hg increase in DBP and retinal vascular diameter.

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Table 40. Multivariate Analysis of Association between Blood pressure and Retinal Vascular Caliber

	N	CRAE, µm Mean (SD) or β (SE)	p or p trend	CRVE, µm Mean (SD) or β (SE)	p or p trend
Systolic blood pressure, mm Hg					
1st quartile, ≤102.50	168	125.77 (2.47)	<0.001	167.12 (3.53)	0.58
2nd quartile, 105.51-110.40	166	123.70 (2.44)		168.02 (3.48)	
3rd quartile, 110.41-119.33	164	122.77(2.44)		168.24 (3.49)	
4th quartile, >119.33	165	122.69 (2.44)		167.81 (3.48)	
Each 10 mm Hg increase	665	-0.87 (0.26)	0.001	0.11 (0.38)	0.77
Diastolic blood pressure, mm Hg					
1st quartile, ≤60.37	166	125.38 (2.44)	<0.001	166.60 (3.50)	0.14
2nd quartile, 60.38-66.67	167	123.90 (2.42)		168.06 (3.47)	
3rd quartile, 66.68-73.21	166	123.38 (2.44)		167.71 (3.50)	
4th quartile, >73.21	166	121.27 (2.43)		168.76 (3.48)	
Each 10 mm Hg increase	665	-1.73 (0.34)	<0.001	0.76 (0.50)	0.13
Mean arterial blood pressure, mm Hg					
1st quartile, ≤75.43	166	125.93 (2.45)	<0.001	167.16 (3.49)	0.44
2nd quartile, 75.44-80.60	169	123.85 (2.40)		167.85 (3.51)	
3rd quartile, 80.61-87.82	164	122.37 (2.44)		168.66 (3.45)	
4th quartile, >87.82	166	121.85 (2.43)		168.00 (3.53)	
Each 10 mm Hg increase	665	-1.86 (0.36)	<0.001	0.65 (0.53)	0.22
Pre-eclampsia risk group					
Low risk, MABP ≥ 90 mm Hg	538	125.12 (2.34)	<0.01	167.54 (3.35)	0.77
High risk, MABP < 90 mm Hg	127	122.65 (2.45)		167.19 (3.49)	

The regression coefficient or mean of CRAE or CRVE was adjusted for age, ethnicity, household income, primiparious status, hypertension, diabetes, cigarette smoking, body mass index, spherical equivalent, Edinburgh Postnatal Depression Score (EPDS) and fellow retinal vessel caliber.

Table 41. Multivariate Analysis of Association between Blood pressure and Retinal Vascular Tortuosity

		Tortuosity A. ($x10^{-6}$)		Tortuosity V. ($x10^{-6}$)	
	N	Mean (SD) or β (SE)	p or p trend	Mean (SD) or β (SE)	p or p trend
Systolic blood pressure, mm Hg					
1st quartile, ≤102.50	168	120.29 (9.19)	0.69	115.39 (9.76)	0.58
2nd quartile, 105.51-110.40	166	116.68 (9.13)		114.78 (9.70)	
3rd quartile, 110.41-119.33	164	118.49 (9.10)		116.61 (9.66)	
4th quartile, >119.33	165	118.76 (9.08)		113.41 (9.65)	
Each 10 mm Hg increase	665	-0.15 (0.65)		-0.52 (0.69)	0.45
Diastolic blood pressure, mm Hg					
1st quartile, ≤60.37	166	119.62 (9.16)	0.29	115.03 (9.77)	0.77
2nd quartile, 60.38-66.67	167	117.70 (9.05)		114.53 (9.65)	
3rd quartile, 66.68-73.21	166	119.35 (9.12)		115.43 (9.73)	
4th quartile, >73.21	166	116.61 (9.17)		113.98 (9.78)	
Each 10 mm Hg increase	665	-0.66 (0.84)		-0.16 (0.89)	0.86
Mean arterial blood pressure, mm Hg					
1st quartile, ≤75.43	166	119.08 (9.19)	0.51	115.78 (9.78)	0.69
2nd quartile, 75.44-80.60	169	118.77 (9.13)		114.48 (9.71)	
3rd quartile, 80.61-87.82	164	117.98 (9.12)		115.02 (9.71)	
4th quartile, >87.82	166	117.78 (9.10)		114.60 (9.69)	
Each 10 mm Hg increase	665	-0.59 (0.89)	0.51	-0.44 (0.95)	0.64
Pre-eclampsia risk group					
Low risk, MABP ≥ 90 mm Hg	538	117.87 (9.26)	0.88	110.37 (10.45)	0.29
High risk, MABP < 90 mm Hg	127	118.76 (9.83)		117.08 (9.84)	

The regression coefficient or mean of retinal vascular tortuosity was adjusted for age, ethnicity, household income, primiparious status, hypertension, diabetes, cigarette smoking, body mass index and spherical equivalent.

Table 42. Multivariate Analysis of Association between Blood pressure and Retinal Vascular Branching Angle

	N	Branching angle A. Degree Mean (SD) or β (SE)	p or p trend	Branching angle V. Degree Mean (SD) or β (SE)	p or p trend
Systolic blood pressure, mm Hg					
1st quartile, ≤102.50	168	87.56 (4.85)	<0.01	74.28 (4.71)	0.42
2nd quartile, 105.51-110.40	166	86.51 (4.82)		75.24 (4.68)	
3rd quartile, 110.41-119.33	164	84.81 (4.80)		75.74 (4.66)	
4th quartile, >119.33	165	84.63 (4.79)		75.08 (4.65)	
Each 10 mm Hg increase	665	-0.75 (0.34)	0.03	0.36 (0.33)	0.29
Diastolic blood pressure, mm Hg					
1st quartile, ≤60.37	166	85.48 (4.87)	0.29	74.52 (4.69)	0.07
2nd quartile, 60.38-66.67	167	85.28 (4.80)		75.15 (4.63)	
3rd quartile, 66.68-73.21	166	84.64 (4.85)		75.80 (4.67)	
4th quartile, >73.21	166	84.38 (4.87)		76.45 (4.69)	
Each 10 mm Hg increase	665	-0.60 (0.44)	0.18	0.86 (0.43)	0.05
Mean arterial blood pressure, mm Hg					
1st quartile, ≤75.43	166	87.70 (4.84)	0.01	74.62 (4.70)	0.29
2nd quartile, 75.44-80.60	169	85.25 (4.81)		74.73 (4.67)	
3rd quartile, 80.61-87.82	164	85.19 (4.80)		75.89 (4.66)	
4th quartile, >87.82	166	84.49 (4.79)		75.53 (4.65)	
Each 10 mm Hg increase	665	-0.92 (0.47)	0.05	0.87 (0.46)	0.38
Pre-eclampsia risk group					
Low risk, MABP ≥ 90 mm Hg	538	82.96 (4.78)	0.10	77.87 (4.63)	0.82
High risk, MABP < 90 mm Hg	127	81.18 (4.77)		77.64 (4.62)	

The regression coefficient or mean of retinal vascular branching angle was adjusted for age, ethnicity, household income, primiparious status, hypertension, diabetes, cigarette smoking, body mass index and spherical equivalent.

Table 43. Multivariate Analysis of Association between Blood pressure and Retinal Vascular Fractal Dimension

		Fractal dimension A. Df ($x10^{-3}$)		Fractal dimension V. Df ($x10^{-3}$)	
	N	Mean (SD) or β (SE)	p or p trend	Mean (SD) or β (SE)	p or p trend
Systolic blood pressure, mm Hg					
1st quartile, ≤102.50	168	125.64 (1.63)	0.04	122.95 (1.48)	0.29
2nd quartile, 105.51-110.40	166	124.42 (1.61)		123.15 (1.46)	
3rd quartile, 110.41-119.33	164	124.57 (1.61)		123.22 (1.46)	
4th quartile, >119.33	165	124.34 (1.61)		123.52 (1.46)	
Each 10 mm Hg increase	665	-4.41 (1.73)	0.01	0.78 (1.57)	0.62
Diastolic blood pressure, mm Hg					
1st quartile, ≤60.37	166	125.26 (1.62)	<0.01	123.25 (1.47)	0.93
2nd quartile, 60.38-66.67	167	124.87 (1.60)		123.25 (1.45)	
3rd quartile, 66.68-73.21	166	124.36 (1.62)		123.25 (1.47)	
4th quartile, >73.21	166	123.82 (1.62)		123.30 (1.47)	
Each 10 mm Hg increase	665	-5.56 (2.23)	0.01	1.42 (2.02)	0.48
Mean arterial blood pressure, mm Hg					
1st quartile, ≤75.43	166	125.41 (1.63)	0.02	123.26 (1.47)	0.51
2nd quartile, 75.44-80.60	169	124.75 (1.60)		122.97 (1.45)	
3rd quartile, 80.61-87.82	164	124.30 (1.62)		123.69 (1.47)	
4th quartile, >87.82	166	124.04 (1.62)		123040 (1.46)	
Each 10 mm Hg increase	665	-6.95 (2.37)	<0.01	1.56 (2.15)	0.47
Pre-eclampsia risk group					
Low risk, MABP ≥ 90 mm Hg	538	123.89 (1.96)	0.07	124.37 (1.77)	0.86
High risk, MABP < 90 mm Hg	127	122.91 (1.97)		124.46 (1.78)	

The regression coefficient or mean of retinal vascular fractal dimension was adjusted for age, ethnicity, household income, primiparious status, hypertension, diabetes, cigarette smoking, body mass index and spherical equivalent.

Table 40-43 show the associations between blood pressure measures and retinal vascular parameters among 665 pregnant women. After adjusting for age, ethnicity, household income, pregnancy outcome history, hypertension history, diabetes history, cigarette smoking history, BMI, spherical equivalent, EPDS and fellow vessel caliber (Model 2), every 10 mmHg increase in SBP, DBP and MABP, statistically significant reductions of 0.9 µm (95% Confidence Interval [CI]: -1.5 µm, -0.4 µm), 1.7 µm (95% CI: -2.5 µm, -1.1 µm) and 1.9 µm (95% CI: -2.86 µm, -1.3 µm) were observed in retinal arteriolar caliber, respectively. Similar linear associations of higher systolic blood pressure ($p=0.02$), higher diastolic blood pressure ($p<0.001$) and higher mean arterial blood pressure ($p<0.001$) with smaller AVR were observed. Furthermore, there was a significant decreasing trend (all p value <0.001) in retinal arteriolar caliber from the lowest quartile to highest quartile in SBP, DBP and MABP (**Table 43**).

There were consistent associations of higher SBP, higher DBP and higher MABP with smaller retinal arteriolar branching angle and smaller retinal arteriolar fractal dimension. Similarly, there were significant descending trends found in retinal arteriolar branching angle and retinal arteriolar fractal dimension, when we compared the highest quartiles of all blood pressure types with the lowest quartiles (**Table 42-43**). However, there was no association between blood pressure measurements and retinal vascular curvature tortuosity (**Table 41**).

3.4.2.1.2 Anthropometric Parameters as Risk Factors for Changes in Retinal Vascular Parameters

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Table 44. Retinal Vascular Calibre by Quartiles of Weight, Height, BMI and TSF in Different Models

		CRAE, µm		CRVE, µm	
	N	Mean (SD) or β (95% CI)	p or p trend	Mean (SD) or β (95% CI)	p or p trend
Weight, kg					
1st quartile, ≤26.58	34	160.76 (14.85)	0.30	222.28 (21.55)	0.18
2nd quartile, 26.59-34.85	34	154.74 (14.81)		215.94 (23.18)	
3rd quartile, 34.86-47.50	34	156.26 (13.50)		218.09 (18.81)	
4th quartile, >47.50	34	152.69 (15.05)		224.49 (21.85)	
Each SD (13.74 kg) increase	136	1.82 (-4.46, 8.10)	0.57	0.09 (-8.58, 8.75)	0.99
Height, cm					
1st quartile, ≤128.98	34	164.20 (15.29)	0.46	225.96 (22.57)	0.51
2nd quartile, 128.99-141.50	34	154.31 (13.68)		212.93 (21.23)	
3rd quartile, 141.51-155.30	34	151.70 (11.24)		220.09 (17.59)	
4th quartile, >155.30	34	153.63 (16.33)		222.73 (22.82)	
Each SD (17.09 cm) increase	136	-1.38 (-6.77, 4.01)	0.62	3.03 (-4.40, 10.45)	0.42
Body mass index, kg/m^2					
1st quartile, ≤15.19	34	160.76 (14.85)	0.12	223.91 (21.90)	0.03
2nd quartile, 15.20-16.84	34	154.74 (14.81)		218.47 (22.88)	
3rd quartile, 16.85-20.16	34	156.26 (13.50)		223.62 (22.18)	
4th quartile, >20.16	34	152.69 (15.05)		220.16 (22.18)	
Each SD (3.52 kg/m^2) increase	136	-0.64 (-2.34, 1.06)	0.46	3.40 (1.04, 5.76)	<0.01

	N	CRAE, µm Mean (SD) or β (95% CI)	p or p trend	CRVE, µm Mean (SD) or β (95% CI)	p or p trend
BMI threshold					
Below	101	155.59 (15.42)	0.74	218.05 (20.54)	0.02
Above	35	155.60 (13.08)		227.38 (22.98)	
Triceps skinfold, mm					
1st quartile, ≤10.07	34	160.76 (14.85)	0.18	212.03 (22.71)	<0.01
2nd quartile, 10.08-12.57	34	154.74 (14.81)		222.27 (17.32)	
3rd quartile, 12.58-15.55	34	156.26 (13.50)		220.94 (20.91)	
4th quartile, >15.55	34	152.69 (15.05)		225.12 (21.71)	
Each SD (4.49 mm) increase	136	-0.98 (-2.63, 0.66)	0.24	2.94 (0.65, 5.23)	0.01
Triceps skinfold threshold					
Below	101	156.53 (15.74)	0.05	217.75 (20.59)	0.001
Above	35	152.88 (10.98)		227.96 (22.44)	

The regression coefficient or mean of retinal vascular caliber was adjusted for age, gender, father's education, systolic blood pressure, birth weight, axial length and fellow retinal vessel.

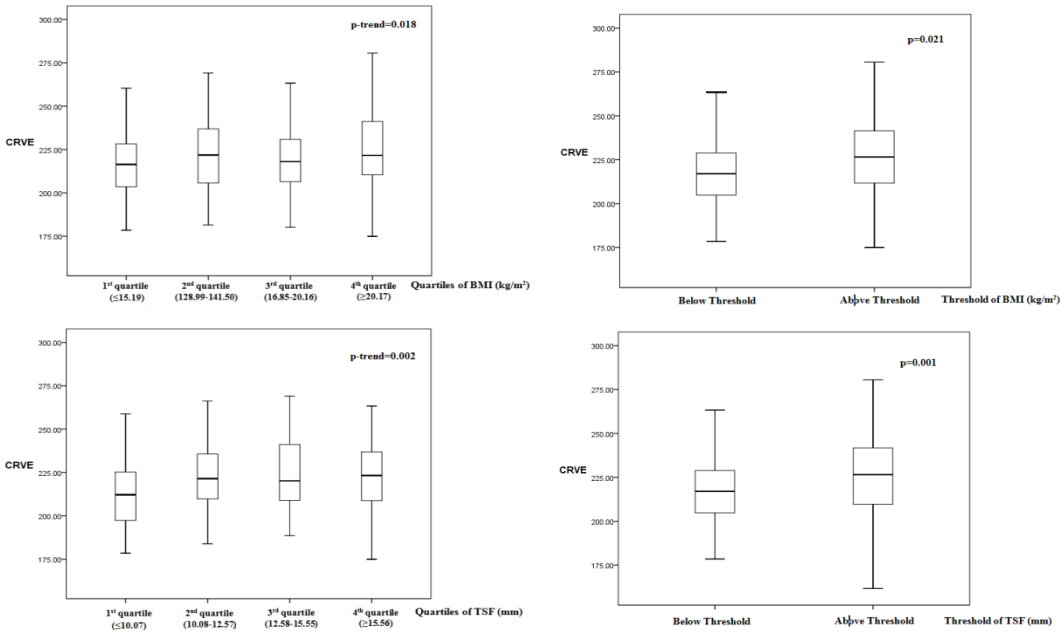


Figure 22. Box Plots of Association between Categories of BMI and TSF with Retinal Venular Caliber.

The mean retinal arteriolar and venular calibre in children by quartiles of height, weight, BMI and TSF were shown in three different models in **Table 49**. In multivariate analysis, the mean retinal venular calibre was 216.48±21.42-µm in the lowest quartile and 225.44±24.63-µm in the highest quartile of BMI (p-trend=0.02), and 212.03±22.71-µm in the lowest quartile and 225.12±21.71-µm in the highest quartile of TSF (p-trend<0.01), and (**Figure 23**).

Based on the TSF and BMI American standard charts to define for age- and gender- specific children overweight and obesity, we found a 9.54-µm increase in mean venular calibre in our children with TSF above threshold compared to those with TSF below the threshold (p<0.01), while a 8.67-µm increase in mean venular calibre in the children with BMI above the threshold compared to children with BMI below the threshold (p=0.03) (**Table 44** and **Figure 23**).

In multiple linear regression models, there was significant retinal venular widening for each SD increase in both TSF and BMI. After adjustment for age, gender, father's education, SBP, birth weight, axial length and fellow vessel, there was a 2.94-µm (95% CI, 0.65 to 5.23; p=0.01) increase and a 3.40-µm (95% CI, 1.04 to 5.76; p<0.01) increase in retinal venular calibre for each SD increase in TSF (4.49 mm) and BMI (3.52 kg/m^2) , respectively.

B. GUSTO retinal study

Table 45. Multiple Linear Regression Analysis of 26 Weeks Gestational and Pre-pregnancy Body Mass Index (BMI) and Weight Gain with Retinal Vascular Calibre

	N	CRAE, µm β/Mean (SE)	p or p trend	CRVE, µm β/Mean (SE)	p or p trend
Pre-pregnancy BMI, kg/m^2					
WHO guideline for BMI categories					
Underweight, ≤18.49	82	124.87 (2.90)	<0.001	169.83 (4.08)	<0.01
Normal weight, 18.50-25.00	462	123.38 (2.74)		173.01 (3.85)	
Overweight, 25.01-29.99	135	119.86 (2.78)		176.04 (3.89)	
Obese, ≥30.00	62	118.81 (2.73)		175.81 (3.82)	
Each SD (4.61 kg/m^2) increase*	741	-0.87 (0.26)	0.001	0.11 (0.38)	0.77
26 weeks pregnancy BMI, kg/m^2					
1st quartile, ≤23.15	140	124.14 (2.79)	<0.001	171.98 (3.92)	0.03
2nd quartile, 23.16-25.54	149	123.54 (2.80)		172.74 (3.93)	
3rd quartile, 25.55-28.81	152	120.92 (2.72)		174.28 (3.82)	
4th quartile, >28.81	143	120.36 (2.68)		175.17 (3.77)	
Each SD (4.57 kg/m^2) increase*	814	-1.58 (0.38)	<0.001	1.28 (0.54)	0.02
Weight gain, kg					
IOM guideline categories					
Less than ideal weight gain	504	123.17 (2.71)	0.05	172.91 (3.80)	0.24
Within ideal weight gain	157	121.91 (2.77)		172.78 (3.89)	
Greater than ideal weight gain	80	120.68 (2.83)		175.08 (3.97)	
Each SD (12.30 kg) increase‡	741	-0.62 (0.37)	0.10	0.54 (0.53)	0.31

*Adjusted for age, ethnicity, primiparious status, household income, hypertension, diabetes, cigarette smoking, systolic blood pressure, spherical equivalent, Pittsburgh Sleep Quality Index (PSQI), weight gain and fellow vessel.

‡Adjusted for age, ethnicity, primiparious status, household income, hypertension, diabetes, cigarette smoking, systolic blood pressure, spherical equivalent, Pittsburgh Sleep Quality Index (PSQI), mean of sum of pre-pregnancy weight and 26 weeks gestational weight and fellow vessel.

Table 46. Multiple Linear Regression Analysis of 26 Weeks Gestational and Pre-pregnancy Body Mass Index (BMI) and Weight Gain with Retinal Vascular Tortuosity

		Tortuosity A., ($x10^{-6}$)		Tortuosity V., ($x10^{-6}$)	
	N	β (SE) or Mean (SE)	p or p trend	β (SE) or Mean (SE)	p or p trend
Pre-pregnancy BMI, kg/m^2					
WHO guideline for BMI categories					
Underweight, ≤18.49	82	111.15 (7.56)	0.77	117.07 (7.30)	<0.01
Normal weight, 18.50-25.00	462	111.56 (7.13)		121.49 (6.89)	
Overweight, 25.01-29.99	135	109.32 (7.24)		124.19 (7.00)	
Obese, ≥30.00	62	110.52 (7.10)		129.32 (6.86)	
Each SD (4.61 kg/m^2) **i**ncrease*	741	-0.79 (0.99)	0.43	2.57 (0.96)	<0.01
26 weeks pregnancy BMI, kg/m^2					
1st quartile, ≤23.15	140	113.10 (7.20)	0.19	122.27 (7.02)	0.10
2nd quartile, 23.16-25.54	149	113.43 (7.22)		123.51 (7.05)	
3rd quartile, 25.55-28.81	152	111.28 (7.03)		123.77 (6.86)	
4th quartile, >28.81	143	109.77 (6.93)		127.15 (6.76)	
Each SD (4.57 kg/m^2) increase*	814	-0.84 (0.98)	0.39	2.44 (0.95)	0.01
Weight gain, kg					
IOM guideline categories					
Less than ideal weight gain	504	112.33 (7.00)	0.37	123.07 (6.75)	0.42
Within ideal weight gain	157	123.27 (5.35)		123.52 (6.92)	
Greater than ideal weight gain	80	118.67 (4.60)		120.44 (7.06)	
Each SD (12.30 kg) increase‡	741	-0.54 (0.71)	0.45	-0.47 (0.93)	0.61

*Adjusted for age, ethnicity, primiparious status, household income, hypertension, diabetes, cigarette smoking, systolic blood pressure, spherical equivalent, weight gain and Pittsburgh Sleep Quality Index (PSQI).

‡Adjusted for age, ethnicity, primiparious status, household income, hypertension, diabetes, cigarette smoking, systolic blood pressure, spherical equivalent, Pittsburgh Sleep Quality Index (PSQI) and mean of sum of pre-pregnancy weight and 26 weeks gestational weight.

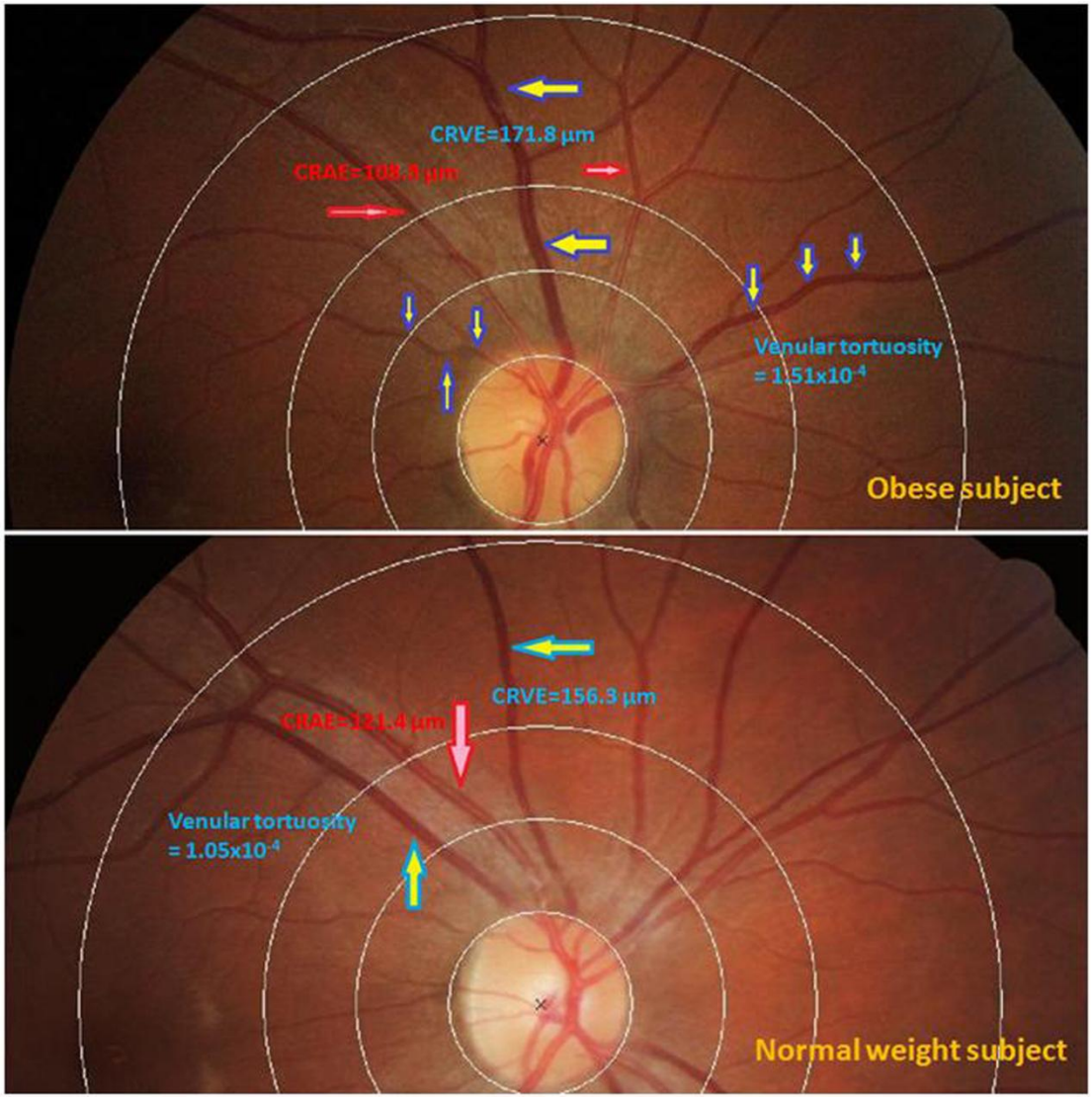


Figure 23. Examples of retinal fundus photographs from an obese subject (upper panel) and a normal weight subject (lower panel). Blue arrows filled with yellow color are indicating retinal venules while red arrows filled with pink color are indicating retinal arterioles. Central retinal arteriolar equivalent (CRAE), central retinal venular equivalent (CRVE) and retinal venular tortuosity are shown in each image.

Table 47. Multiple Linear Regression Analysis of 26 Weeks Gestational and Pre-pregnancy Body Mass Index (BMI) and Weight Gain with Retinal Vascular Branching Angle

		Branching angle A., degree		Branching angle V., degree	
	N	β (SE) or Mean (SE)	p or p trend	β (SE) or Mean (SE)	p or p trend
Pre-pregnancy BMI, kg/m^2					
WHO guideline for BMI categories					
Underweight, ≤18.49	82	82.46 (3.91)	0.97	84.80 (3.79)	0.43
Normal weight, 18.50-25.00	462	83.75 (3.54)		82.48 (3.43)	
Overweight, 25.01-29.99	135	81.48 (3.62)		83.97 (3.51)	
Obese, ≥30.00	62	83.10 (3.55)		86.51 (3.44)	
Each SD (4.61 kg/m^2) increase*	741	-0.25 (0.62)	0.68	1.05 (0.60)	0.08
26 weeks pregnancy BMI, kg/m^2					
1st quartile, ≤23.15	140	84.71 (3.61)	0.24	83.37 (3.51)	0.27
2nd quartile, 23.16-25.54	149	84.13 (3.58)		84.92 (3.49)	
3rd quartile, 25.55-28.81	152	81.92 (3.48)		83.95 (3.39)	
4th quartile, >28.81	143	83.26 (3.46)		85.68 (3.37)	
Each SD (4.57 kg/m^2) increase*	814	-0.32 (0.61)	0.60	1.03 (0.59)	0.08
Weight gain, kg					
IOM guideline categories					
Less than ideal weight gain	504	82.68 (3.48)	0.56	84.93 (3.36)	0.21
Within ideal weight gain	157	83.00 (3.55)		83.41 (3.43)	
Greater than ideal weight gain	80	83.80 (3.72)		82.61 (3.59)	
Each SD (12.30 kg) increase‡	741	0.07 (0.55)	0.90	-0.21 (0.54)	0.69

*Adjusted for age, ethnicity, primiparious status, household income, hypertension, diabetes, cigarette smoking, systolic blood pressure, spherical equivalent, weight gain and Pittsburgh Sleep Quality Index (PSQI).

‡Adjusted for age, ethnicity, primiparious status, household income, hypertension, diabetes, cigarette smoking, systolic blood pressure, spherical equivalent, Pittsburgh Sleep Quality Index (PSQI) and mean of sum of pre-pregnancy weight and 26 weeks gestational weight.

Table 48. Multiple Linear Regression Analysis of 26 Weeks Gestational and Pre-pregnancy Body Mass Index (BMI) and Weight Gain with Retinal Vascular Fractal Dimension

		Fractal dimension A., Df		Fractal dimension V., Df	
	N	β (SE) or Mean (SE)	p or p trend	β (SE) or Mean (SE)	p or p trend
Pre-pregnancy BMI, kg/m^2					
WHO guideline for BMI categories					
Underweight, ≤18.49	82	124.8 (2.15)	0.19	120.7 (1.84)	0.10
Normal weight, 18.50-25.00	462	124.3 (1.95)		122.7 (1.67)	
Overweight, 25.01-29.99	135	124.1 (2.00)		122.7 (1.71)	
Obese, ≥30.00	62	122.8 (1.96)		122.9 (1.68)	
Each SD (4.61 kg/m^2) increase*	741	7.31 (5.06)	0.15	7.18 (4.37)	0.10
26 weeks pregnancy BMI, kg/m^2					
1st quartile, ≤23.15	140	124.5 (1.99)	0.18	122.3 (1.70)	0.68
2nd quartile, 23.16-25.54	149	124.4 (1.98)		123.3 (1.69)	
3rd quartile, 25.55-28.81	152	123.5 (1.19)		122.0 (1.64)	
4th quartile, >28.81	143	123.4 (1.91)		123.2 (1.63)	
Each SD (4.57 kg/m^2) increase*	814	-0.60 (0.33)	0.07	0.10 (0.29)	0.74
Weight gain, kg					
IOM guideline categories					
Less than ideal weight gain	504	124.3 (1.53)	0.22	121.7 (1.31)	0.93
Within ideal weight gain	157	123.9 (1.64)		121.6 (1.40)	
Greater than ideal weight gain	80	123.0 (1.72)		121.6 (1.47)	
Each SD (12.30 kg) increase‡	741	-0.15 (0.31)	0.63	-0.18 (0.26)	0.50

*Adjusted for age, ethnicity, primiparious status, household income, hypertension, diabetes, cigarette smoking, systolic blood pressure, spherical equivalent, weight gain and Pittsburgh Sleep Quality Index (PSQI).

‡Adjusted for age, ethnicity, primiparious status, household income, hypertension, diabetes, cigarette smoking, systolic blood pressure, spherical equivalent, Pittsburgh Sleep Quality Index (PSQI) and mean of sum of pre-pregnancy weight and 26 weeks gestational.

Table 49. Multivariate Analysis of 26 Weeks Gestational Subcutaneous Skinfold Thickness and Retinal Vascular Caliber

		CRAE, µm		CRVE, µm	
26 weeks'	**N**	**β (SE) or Mean (SE)**	**p or p trend**	**β (SE) or Mean (SE)**	**p or p trend**
Triceps skinfold, mm					
1st quartile, ≤18.80	200	124.53 (3.02)	0.07	168.49 (4.20)	0.47
2nd quartile, 18.81-22.87	196	122.75 (2.93)		170.29 (4.07)	
3rd quartile, 22.88-27.13	195	124.36 (3.03)		166.18 (4.20)	
4th quartile, >27.13	200	121.64 (2.93)		171.17 (4.06)	
Each SD (6.06 mm) **i**ncrease	813	-4.40 (2.36)	0.06		0.40
Biceps skinfold, mm					
1st quartile, ≤8.45	199	122.12 (3.01)	0.40	168.52 (4.28)	0.13
2nd quartile, 8.46011.40	198	122.08 (2.93)		169.71 (4.18)	
3rd quartile, 11.41-15.72	197	120.62 (2.91)		171.84 (4.13)	
4th quartile, >15.72	200	121.51 (2.88)		170.55 (4.12)	
Each SD (5.39 mm) **i**ncrease	814	-0.07 (0.43)	0.88	0.89 (0.59)	0.13
Subscalpular skinfold, mm					
1st quartile, ≤17.60	199	121.89 (2.93)	0.29	170.63 (4.16)	0.54
2nd quartile, 17.61-22.47	199	121.97 (2.92)		168.83 (4.22)	
3rd quartile, 22.48-27.50	196	120.09 (2.94)		171.70 (4.18)	
4th quartile, >27.50	199	121.19 (2.88)		170.75 (4.14)	
Each SD (6.50 mm) **i**ncrease	813	-0.32 (0.43)	0.45	0.45 (0.60)	0.45
Suprailiac skinfold, mm					
1st quartile, ≤19.75	200	123.38 (3.02)	0.26	169.56 (4.22)	0.10
2nd quartile, 19.76-23.73	196	122.37 (2.97)		169.54 (4.15)	
3rd quartile, 23.74-27.73	194	122.45 (3.05)		170.30 (4.25)	
4th quartile, >27.73	194	121.96 (2.97)		172.24 (4.14)	
Each SD (5.59 mm) **i**ncrease	812	-0.47 (0.43)	0.28	0.93 (0.60)	0.12

The regression coefficient or mean of CRAE or CRVE were adjusted for age, ethnicity, primiparious status, household income, hypertension, diabetes, cigarette smoking, systolic blood pressure, spherical equivalent, Pittsburgh Sleep Quality Index (PSQI) and fellow vessel.

Table 50. Multivariate Analysis of 26 Weeks Gestational Subcutaneous Skinfold Thickness and Other Retinal Vascular Parameters

Each SD increase in 26 weeks' subcutaneous skinfold thickness	N	Retinal vascular geometric parameters β (SE)	p	β (SE)	p
		Tortuosity A., x10^{-6}		**Tortuosity V., x10^{-6}**	
Triceps skinfold, 6.06mm	813	4.77 (6.98)	0.50	-2.94 (7.24)	0.69
Biceps skinfold, 5.39mm	814	0.34 (1.33)	0.80	-1.25 (1.38)	0.37
Subscalpular skinfold, 6.50mm	812	1.36 (1.37)	0.32	0.09 (1.42)	0.95
Suprailiac skinfold, 5.59mm	812	0.32 (1.23)	0.80	-0.27 (1.23)	0.84
		Branching angle A., D		**Branching angle V., D**	
Triceps skinfold, 6.06mm	813	-2.60 (3.66)	0.48	-0.48 (3.52)	0.89
Biceps skinfold, 5.39mm	814	-0.91 (0.70)	0.19	-0.62 (0.67)	0.36
Subscalpular skinfold, 6.50mm	812	-0.71 (0.72)	0.32	-0.57 (0.69)	0.42
Suprailiac skinfold, 5.59mm	812	-1.04 (0.64)	0.11	-0.21 (0.62)	0.73
		Fractal dimension A., x10^{-3}		**Fractal dimension V., x10^{-3}**	
Triceps skinfold, 6.06mm	813	0.19 (0.25)	0.47	0.14 (0.27)	0.98
Biceps skinfold, 5.39mm	814	0.44 (0.42)	0.28	0.19 (0.36)	0.49
Subscalpular skinfold, 6.50mm	812	0.48 (0.38)	0.70	-0.05 (0.34)	0.88
Suprailiac skinfold, 5.59mm	812	-0.02 (0.34)	0.95	0.04 (0.30)	0.89

The regression coefficient or mean of CRAE or CRVE were adjusted for age, ethnicity, primiparious status, household income, hypertension, diabetes, cigarette smoking, systolic blood pressure, spherical equivalent and Pittsburgh Sleep Quality Index (PSQI).

Table 45-50 shows the associations between anthropometric measurements and retinal vascular parameters among pregnant women. The associations between maternal BMI (pre-pregnancy and pregnancy) and retinal vascular caliber are shown in **Table 45**. The associations of higher pre-pregnancy and 26-week pregnancy BMI with retinal arteriolar narrowing and retinal venular widening were consistent in a multivariable adjusted model. In the multivariable model, each SD increase of pre-pregnancy BMI (4.61 kg/m^2) was associated with a reduction of 1.61 µm ($p<0.001$) in retinal arteriolar caliber and an increase of 1.26 µm ($p=0.02$) in retinal venular caliber. Similarly, each SD increase in 26 week pregnancy BMI (4.57 kg/m^2) was associated with a reduction of 1.58 µm ($p<0.001$) in retinal arteriolar caliber and an increase of 1.28 µm ($p=0.02$) in retinal venular caliber. Women in the highest quartile of pregnancy BMI had significantly narrower retinal arteriolar caliber (120.36 vs. 124.14 µm; p trend<0.001) and a wider retinal venular caliber (175.17 vs. 171.98 µm; p trend=0.03), compared with the lowest quartile of pregnancy BMI. Obese and overweight pregnant women had narrower arteriolar caliber (118.81/119.86 vs. 124.87/123.38 µm; p trend <0.001) and wider retinal venular caliber (175.81/176.04 vs. 169.83/173.01 µm; p trend <0.01) compared with women who were underweight and normal weight.

Table 45 also shows the association between pregnancy weight gain and retinal vascular caliber. In multiple linear regression model, pregnancy weight gain was not associated with retinal arteriolar caliber (-0.62 µm; p=0.10) or retinal venular caliber (0.15 µm; p=0.31) Women with greater weight gain regardless of pre-pregnancy BMI

status had marginally narrower retinal arteriolar caliber than those with normal or lower weight gain (120.68 vs. 121.91 vs. 123.17 μm; p trend=0.05).

In **Table 46**, each SD increase of pregnancy and pre-pregnancy BMI was associated with an increase of 2.44 (10^{-6}) (p=0.01) and 2.57 (10^{-6}) (p<0.01) in retinal venular tortuosity, respectively. Obese subjects had the largest retinal venular tortuosity compared with the other three groups (129.92 vs. 124.19-117.07 [$10^{-}6$]; p<0.01). No association was found between weight gain and retinal arteriolar tortuosity ($-1.10x10^{-6}$, p=0.26) or retinal venular tortuosity ($-0.47x10^{-6}$, p=0.61).

Figure 24 shows the visual differences of retinal arteriolar caliber, retinal venular caliber and retinal venular tortuosity between an obese subject and a normal weight subject who were selected from our study. The chosen obese subject has a narrower CRAE (108.3 vs. 121.4 μm), a wider CRVE (171.8 vs. 156.3 μm) and a more tortuous retinal venule ($1.51x10^{-4}$ vs. $1.05x10^{-4}$) compared with the chosen normal subject.

No statistically significant associations were observed between pre-pregnancy BMI, pregnancy BMI and weight gain and retinal vascular branching angle and retinal vascular fractal dimension (**Table 47-48**).

For subcutaneous skinfold thickness measurements, triceps, biceps, subscapular and suprailiac skinfold were assessed and analyzed accordingly. However, no association between any type of these skinfold thicknesses and retinal vascular parameters was found by using multivariate analysis.

3.4.2.1.3 Ocular Biometric Parameters as Risk Factors for Changes in Retinal Vascular Parameters

A. STARS and STARS Family retinal study

Table 51. Multiple Linear Regression of Association between Corrected Retinal Vascular Caliber and Refraction and Ocular Biometric Parameters

	Corrected CRAE, µm*		Corrected CRVE, µm*	
	β (SE)	p	β (SE)	p
Axial length, mm N=442				
Each unit increase, 1.0 mm‡	-3.52 (1.54)	0.02	-5.55 (2.09)	<0.01
Each unit increase, 1.0 mm§	-2.04 (1.46)	0.16	-3.82 (1.97)	0.16
Corneal curvature, mm N=417				
Each unit increase, 1.0 mm‡	-5.85 (3.47)	0.10	-13.79 (4.69)	<0.01
Each unit increase, 1.0 mm§	-2.21 (3.29)	0.50	-10.97 (4.41)	0.01
Anterior chamber depth, mm N=416				
Each unit increase, 1.0 mm‡	4.76 (3.72)	0.20	0.66 (5.10)	0.90
Each unit increase, 1.0 mm§	4.58 (3.46)	0.19	-1.75 (4.76)	0.71

*To correct for ocular magnification on retinal vascular caliber measurements which was caused by telecentric camera and ocular refractive media, we used a correction factor (1-0.0017*SE) described by Bengtsson for our Canon fundus camera.[265]

‡ Adjusted for age, gender, father's education, parental myopia history, mean arterial blood pressure, body mass index, birth weight and right eye spherical equivalent.

§ Adjusted for age, gender, father's education, parental myopia history, mean arterial blood pressure, body mass index, birth weight, right eye spherical equivalent and retinal fellow vessel.

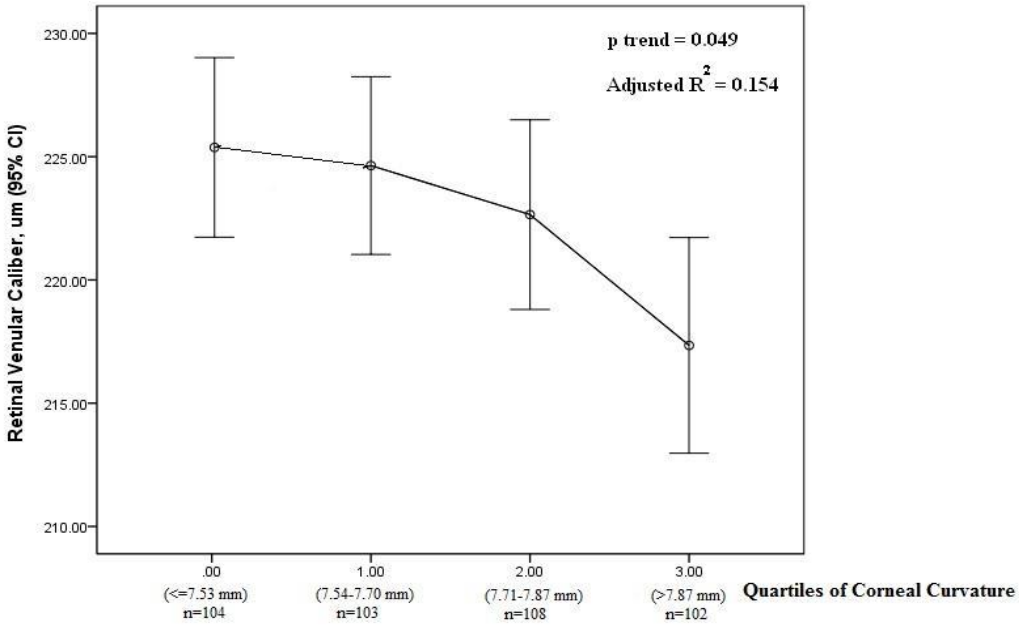


Figure 24. Relationship between Quartiles of Axial Length and Retinal Venular Caliber.

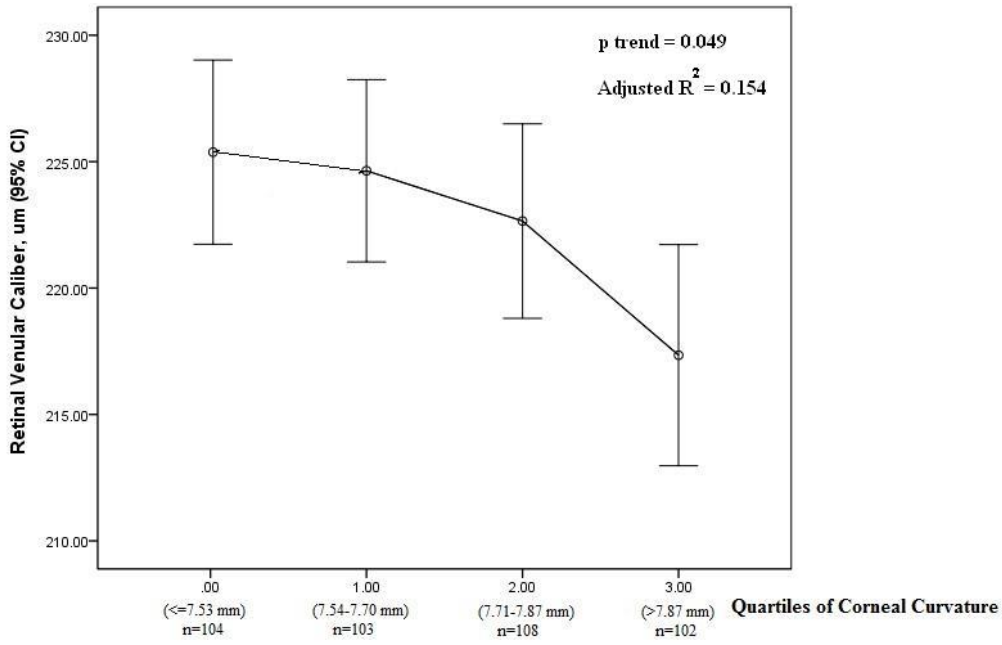


Figure 25. Relationship between Quartiles of Corneal Curvature and Retinal Venular Caliber.

Table 51 describes the association between retinal vascular caliber and ocular biometric parameters. In multiple linear regression models adjusting for age, gender, SE, father's education, parental myopia history, MABP, BMI and birth weight, each 1.0mm increase in axial length was associated with a 3.52μm reduction (p=0.023) in retinal arteriolar caliber and a 5.55μm reduction (p=0.008) in retinal venular caliber. Each 1.0 unit increase in corneal curvature was only associated with 13.79μm reduction (p=0.004) in retinal venular caliber while not with retinal arteriolar caliber (p=0.093). There was no association found between retinal vascular caliber and anterior chamber depth in both linear regression models.

Figure 25-26 shows the association of quartiles of axial length and quartiles of corneal curvature with retinal venular caliber after controlling for age, gender, SE, father's education, parental myopia history, MABP, BMI and birth weight. Error plots demonstrated that highest quartile of axial length was significantly associated with narrower retinal venular caliber than the lowest quartile of axial length (p-trend=0.023; adjusted R^2=0.168) (**Figure 25**). And highest quartile of corneal curvature was associated with narrower retinal venular caliber than the lowest quartile of corneal curvature (p-trend=0.022; adjusted R^2=0.153) (**Figure 26**).

3.4.2.1.4 Antenatal Mental Health Status as a Risk Factor for Changes in Retinal Vascular Parameters

Table 52. Multiple Linear Regression of Associations between Antenatal Depression Assessment and Retinal Vascular Caliber among Pregnant Women

		CRAE, µm		CRVE, µm	
Antenatal depression assessment	**N**	**Mean (SE) or β (SE)**	**p or p trend**	**Mean (SE) or β (SE)**	**p or p trend**
EPDS, each SD increase (4.49)	786	0.80 (0.39)	0.03	1.06 (0.54)	0.05
EPDS score in quintiles					
1st quintile, ≤4.0	180	121.11 (1.80)	0.03	168.37 (2.55)	0.04
2nd quintile, 4.1-7.0	198	121.52 (1.75)		169.74 (2.49)	
3rd quintile, 7.1-9.0	131	121.74 (1.84)		170.33 (2.61)	
4th quintile, 9.1-14.0	154	122.20 (1.74)		170.49 (2.47)	
5th quintile, <14.0	123	123.75 (1.86)		171.87 (2.47)	
EPDS antenatal clinical cut-off					
Non-referral depression, <14	688	121.67 (1.27)	0.04	169.72 (4.00)	0.15
Doc-referral depression, ≥14	98	123.91 (2.82)		172.18 (1.81)	
BDI-II, each SD increase (6.64)	771	0.20 (0.40)	0.62	0.61 (0.56)	0.28
BDI-II score in quintiles					
1st quintile, ≤4.0	211	122.19 (1.81)	0.31	169.33 (2.54)	0.30
2nd quintile, 4.1-6.0	120	121.24 (1.93)		170.67 (2.71)	
3rd quintile, 6.1-9.0	147	120.88 (1.84)		169.35 (2.59)	
4th quintile, 9.1-14.0	170	121.33 (1.76)		168.92 (2.47)	
5th quintile, <14.0	123	123.51 (1.81)		172.13 (2.54)	
BDI-II clinical cut-off					
Minimal, ≤13,	316	121.52 (1.71)	0.58	169.05 (2.39)	0.95
Mild, 14-19	102	122.64 (1.85)		170.99 (2.59)	
Moderate, 20-28	43	123.16 (2.25)		173.73 (3.15)	
Severe, ≥29	13	123.26 (3.64)		168.43 (5.09)	

Adjusted for age, ethnicity, household income, primiparous status, hypertension, diabetes, smoking history, MABP, BMI and SE.

Table 53. Multiple Linear Regression of Associations between State-Trait Anxiety Inventory (STAI) and Retinal Vascular Caliber among Pregnant Women

		CRAE, µm		CRVE, µm	
STAI score	**N**	**Mean (SE) or β (SE)**	**p or p trend**	**Mean (SE) or β (SE)**	**p or p trend**
STAI state-anxiety,					
Each 1.0 score increase	781	0.05 (0.04)	0.18	0.04 (0.05)	0.45
STAI state-anxiety					
1st quintile, ≤26.0	164	121.00 (1.86)	0.13	168.68 (2.65)	0.39
2nd quintile, 26.1-32.0	154	121.42 (1.83)		169.88 (2.60)	
3rd quintile, 32.1-38.0	175	121.59 (1.77)		170.55 (2.51)	
4th quintile, 38.1-45.0	142	123.12 (1.80)		170.53 (2.56)	
5th quintile, <45.0	146	122.11 (1.77)		169.95 (2.52)	
STAI trait-anxiety,					
Each 1.0 score increase	776	0.05 (0.04)	0.26	0.04 (0.05)	0.15
1st quintile, ≤29.0	157	121.66 (1.83)		168.67 (2.61)	0.14
2nd quintile, 29.1-35.0	174	121.48 (1.7)		170.30 (2.52)	
3rd quintile, 35.1-40.0	166	120.79 (1.81)		168.15 (2.58)	
4th quintile, 40.1-45.6	124	122.04 (1.80)		169.86 (2.56)	
5th quintile, <45.6	155	123.18 (1.77)		171.61 (2.52)	
STAI total score,					
Each 1.0 score increase	784	0.02 (0.02)	0.33	0.02 (0.03)	0.35
1st quintile, ≤29.0	161	122.04 (1.85)	0.20	169.92 (2.63)	0.26
2nd quintile, 29.1-35.0	153	120.25 (1.86)		168.64 (2.64)	
3rd quintile, 35.1-40.0	165	121.68 (1.76)		169.03 (2.49)	
4th quintile, 40.1-45.6	152	122.33 (1.79)		171.76 (2.54)	
5th quintile, <45.6	153	122.65 (1.76)		170.42 (2.51)	
STAI clinical cut-off					
Above 75th percentile, ≥86.0	193	121.25 (1.26)	0.42	171.74 (1.78)	0.51
Below 75th percentile, <86.0	591	123.24 (2.80)		169.05 (4.00)	

Adjusted for age, ethnicity, household income, primiparous status, hypertension, diabetes, smoking history, MABP, BMI and SE.

Table 54. Multiple Linear Regression of Associations between Pittsburg Sleep Quality Index (PSQI) and Retinal Vascular Caliber among Pregnant Women

		CRAE, µm		CRVE, µm	
PSQI score	**N**	**Mean (SE) or β (SE)**	**p or p trend**	**Mean (SE) or β (SE)**	**p or p trend**
Each SD increase (2.9)	472	1.22 (0.47)	0.01	0.71 (0.66)	0.29
PSQI in quintiles					
1st quintile, ≤3.0	115	122.88 (2.30)	0.02	171.27 (3.20)	0.27
2nd quintile, 3.1-5.0	136	122.84 (2.15)		172.94 (2.99)	
3rd quintile, 5.1-6.0	67	124.85 (2.44)		175.62 (3.41)	
4th quintile, 6.1-8.0	72	125.18 (2.44)		176.66 (3.40)	
5th quintile, <8.0	80	125.57 (2.39)		171.91 (3.33)	
PSQI clinical cut-off					
Normal sleep quality, ≤5.0	253	122.88 (2.11)	0.01	172.31 (2.97)	0.05
Abnormal sleep 1uality, >5.0	291	125.23 (2.21)		174.80 (3.11)	

Adjusted for age, ethnicity, household income, primiparous status, hypertension, diabetes, smoking history, MABP, BMI and SE.

Table 55. Multiple Linear Regression of Associations between Antenatal Mental Health Assessments and Other Retinal Vascular Parameters among Pregnant Women

Each 1.0 score increase in antenatal mental health	N	Retinal vascular geometric parameters β (SE)	p	β (SE)	p
		Tortuosity A., x10^{-6}		**Tortuosity V., x10^{-6}**	
EPDS	786	-0.08 (0.18)	0.65	-0.11 (0.21)	0.58
BDI-II	771	-0.17 (0.13)	0.19	-0.16 (0.15)	0.27
STAI	784	-0.23 (0.04)	0.60	0.11 (0.05)	0.82
PSQI	472	-0.12 (0.35)	0.72	-0.37 (0.38)	0.33
		Branching angle A., Degree		**Branching angle V., Degree**	
EPDS	786	0.07 (0.10)	0.45	-0.09 (0.09)	0.34
BDI-II	771	0.05 (0.07)	0.48	-0.05 (0.07)	0.48
STAI	784	0.02 (0.02)	0.54	-0.02 (0.02)	0.36
PSQI	472	0.08 (0.18)	0.67	-0.44 (0.17)	0.01
		Fractal dimension A., Df		**Fractal dimension V., Df**	
EPDS	786	0.06 (0.05)	0.48	0.08 (0.03)	0.30
BDI-II	771	0.01 (0.01)	0.72	0.02 (0.04)	0.64
STAI	784	0.01 (0.02)	0.26	0.03 (0.01)	0.80
PSQI	472	0.01 (0.07)	0.74	0.02 (0.05)	0.69

Adjusted for age, ethnicity, household income, primiparous status, hypertension, diabetes, smoking history, MABP, BMI and SE.

Table 52-54 shows the associations between antenatal mental health assessments and retinal vascular caliber. In a multivariate model, per SD increase in EPDS score (4.49 scores) was significantly associated with a 0.80μm larger retinal arteriolar caliber (p=0.03). A similar direction was found for the association between higher quintiles of EPDS total depression score and retinal venular caliber (p-trend=0.03). By using EPDS antenatal cut-off 14, subjects classified into doctor referral depression group didn't show a significant wider retinal arteriolar (p=0.10) or venular (p=0.61) caliber than those classified into non-doctor referral depression group. For the depression score evaluated by BDI-II, no association was found with both retinal arteriolar and venular caliber (**Table 52**). There was no significant correlation between the State-Trait Anxiety Inventory (STAI) state-subscale and retinal arteriolar (p=0.18) or venular caliber (p=0.45) (**Table 53**).

Table 54 showed the multivariate analysis of sleep quality and retinal vascular caliber. Per SD increase in PSQI score (2.90 scores) was associated with 1.22μm a larger retinal arteriolar caliber (p=0.01). A significant association was found in participants with higher PSQI total score quintile (p-trend=0.02) and participants clinically classified into abnormal sleeping quality (p-trend=0.01), compared with participants with lower PSQI total score quintiles and participants clinically classified into normal sleeping quality. There was no association found between PSQI score and retinal venular caliber.

Only increased PSQI was associated with smaller retinal venular branching angle (p=0.01), while no other association was found between antenatal mental health assessment and retinal vascular geometric parameters (**Table 55**).

3.4.2.1.5 Birth Parameters as Risk Factors for Changes in Retinal Vascular Parameters

Table 56. Multiple Linear Regression of Associations between Birth Parameters and Retinal Vascular Caliber among Children

		CRAE, µm		CRVE, µm	
Each SD increase in birth parameters	**N**	**β (SE)**	**p or p trend**	**β (SE)**	**p or p trend**
Birth weight, 467.72 g	435	1.06 (0.75)	0.16	-1.0 (1.0)	0.33
Birth weight clinical cut-off					
Low, <2500 g	39	156.32 (2.33)	0.06	226.58 (3.11)	0.61
Normal, 2500-4000 g	392	159.49 (0.78)		221.80 (1.04)	
High, >4000 g	4	172.27 (8.03)		220.86 (10.75)	
Birth length, 2.29 cm	431	1.0 (0.75)	0.19	-0.18 (1.01)	0.86
Head circumference, 1.55cm	430	0.67 (0.82)	0.42	1.29 (1.10)	0.24
Gestational age, 1.93 weeks	89	0.13 (2.29)	0.95	2.13 (3.62)	0.56
Gestational age cut-off					
Premature, <37 weeks	13	163.39 (4.09)	0.20	211.80 (6.50)	0.21
Full term, ≥37 weeks	76	157.57 (1.70)		220.85 (2.70)	

Adjusted for age, gender, father's education, mean arterial blood pressure, body mass index, axial length and fellow vessel.

As shown in **Table 56**, no association was found between any type of birth parameters and retinal vascular caliber among children by using multiple linear regression. However, children with low birth weight had a borderline trend in getting retinal arteriolar narrowing compared with others (p trend=0.06).

3.4.2.1.6 Life Style as a Risk Factor for Changes in Retinal Vascular Parameters

A. STARS and STARS Family retinal study

Table 57. Multiple Linear Regression of Associations between Parental Life Style and Retinal Vascular Caliber among Children

		CRAE, µm		CRVE, µm	
	N	**β (SE)**	**p trend**	**β (SE)**	**p trend**
Mother smoking status					
Non-smoker	422	159.20 (0.75)	0.35	221.99 (1.00)	0.64
Past smoker	13	163.02 (4.04)		219.44 (5.40)	
Current smoker	19	153.31 (3.32)		225.00 (4.44)	
Father smoking status					
Non-smoker	282	158.69 (0.93)	0.47	221.62 (1.24)	0.91
Past smoker	31	159.36 (1.30)		223.33 (1.72)	
Current smoker	143	160.92 (2.94)		221.18 (3.19)	
Mother smoking during pregnancy					
Yes	8	157.72 (4.95)	0.70	225.17 (6.64)	0.98
No	444	159.22 (0.72)		222.09 (0.97)	
Mother alcohol drinking during pregnancy					
Yes	3	160.75 (8.11)	0.84	222.03 (10.79)	0.99
No	449	159.07 (0.72)		222.17 (0.96)	

Adjusted for age, gender, father's education, mean arterial blood pressure, body mass index, axial length and birth weight.

Table 57 shows no association between parental life style and retinal vascular caliber changes among children.

B. GUSTO retinal study

Table 58. Multiple Linear Regression of Associations between Maternal Life Style and Retinal Vascular Parameters

		Retinal vascular parameters			
	N	**β (SE)**	**p trend**	**β (SE)**	**p trend**
		CRAE, µm		**CRVE, µm**	
Mother smoking status					
Non-smoker	702	121.78 (1.47)	0.51	170.22 (2.11)	0.77
Current or Past smoker	122	122.28 (1.55)		169.89 (2.22)	
Mother alcohol drinking status					
Non-smoker	569	122.05 (1.46)	0.11	170.28 (2.09)	0.28
Current or Past smoker	255	122.33 (1.57)		169.09 (2.25)	
		Tortuosity A., x10^{-6}		**Tortuosity V., x10^{-6}**	
Mother smoking status					
Non-smoker	702	112.96 (3.74)	0.78	119.00 (4.06)	0.24
Current or Past smoker	122	112.42 (3.93)		121.47 (4.27)	
Mother alcohol drinking status					
Non-smoker	569	113.31 (3.69)	0.54	119.69 (4.03)	0.90
Current or Past smoker	255	112.12 (3.98)		119.96 (4.34)	
		Branching angle A., D		**Branching angle V., D**	
Mother smoking status					
Non-smoker	702	81.64 (2.03)	0.17	83.25 (1.92)	0.53
Current or Past smoker	122	83.08 (2.13)		83.88 (2.01)	
Mother alcohol drinking status					
Non-smoker	569	82.02 (2.01)	0.93	83.54 (1.89)	0.57
Current or Past smoker	255	81.92 (2.17)		82.99 (2.04)	

		Retinal vascular parameters			
	N	**β (SE)**	**p trend**	**β (SE)**	**p trend**
	Fractal dimension A., x10^{-3}			**Fractal dimension V., x10^{-3}**	
Mother smoking status					
Non-smoker	702	124.93 (0.99)	0.82	122.03 (0.90)	0.63
Current or Past smoker	122	124.82 (1.04)		122.3 (0.95)	
Mother alcohol drinking status					
Non-smoker	569	125.02 (0.98)	0.77	122.07 (0.91)	0.97
Current or Past smoker	255	124.87 (1.06)		122.05 (0.98)	

Adjusted for age, ethnicity, household income, primiparious status, hypertension, diabetes, mean arterial blood pressure, body mass index and spherical equivalent.

Table 58 shows the association between maternal life style factor including cigarette smoking and alcohol drinking and retinal vascular parameters. Maternal smoking and maternal alcohol drinking was not significantly associated with retinal geometric network in multivariate analysis.

3.4.2.1.7 Gender, Ethnicity, Household Income and Education as Risk Factors for Changes in Retinal Vascular Parameters

A. STARS and STARS Family study

Table 59. Association between Gender, Ethnicity, Household Income and Education and Retinal Vascular Caliber among Children

		CRAE, µm		CRVE, µm	
	N	**β (SE)**	**p trend**	**β (SE)**	**p trend**
Gender					
Boys	289	156.61 (1.04)	0.001	222.58 (1.39)	0.67
Girls	297	161.48 (1.05)		221.71 (1.40)	
Father's education					
≤Secondary school,	135	158.46 (1.32)	0.46	220.90 (1.75)	0.34
O/N level,	85	158.23 (1.61)		220.37 (2.14)	
A levels/diploma,	107	160.21 (1.47)		225.63 (1.95)	
University education,	127	159.29 (1.35)		221.70 (1.79)	
Mother's education					
≤Secondary school,	123	158.07 (1.37)	0.46	222.27 (1.84)	0.88
O/N level,	102	160.80 (1.46)		221.33 (1.96)	
A levels/diploma,	127	160.12 (1.31)		223.11 (1.76)	
University education,	101	156.73 (1.50)		222.12 (2.02)	
Household income					
SGD <1000 per mth	16	155.48 (3.76)	0.27	223.06 (5.02)	0.95
SGD 1000-2999 per mth	99	158.60 (1.53)		220.96 (2.04)	
SGD 3000-4999 per mth	149	159.65 (1.25)		222.56 (1.66)	
SGD ≥5000 per mth	177	159.50 (1.15)		222.87 (1.53)	

Adjusted for age, gender/father's education/household income, mean arterial blood pressure, body mass index, birth weight, axial length and fellow vessel.

Table 59 shows the relationship between demographic status and retinal vascular caliber among children. Boys tended to have narrower retinal arterioles than girls.

B. GUSTO retinal study

Table 60. Association between Gender, Ethnicity, Household Income and Education and Retinal Vascular Parameters among Pregnant Women

		Retinal vascular parameters			
	N	**β (SE)**	**p trend**	**β (SE)**	**p trend**
		CRAE, µm		**CRVE, µm**	
Ethnicity					
Chinese	450	120.09 (1.74)	0.03	169.94 (2.49)	0.65
Malay	235	122.59 (1.82)		172.81 (2.61)	
Indians	139	122.44 (1.92)		169.21 (2.74)	
Household income					
SGD <1000 per mth	162	121.59 (1.82)	0.57	172.18 (2.61)	0.14
SGD 1000-2999 per mth	280	120.61 (1.84)		169.91 (2.64)	
SGD 3000-4999 per mth	209	123.15 (1.85)		171.70 (2.64)	
SGD ≥5000 per mth	173	121.49 (1.89)		168.82 (2.70)	
Maternal education					
Primary school,	38	122.26 (2.40)	0.72	168.37 (3.43)	0.374
Secondary school	232	122.26 (1.88)		172.36 (2.68)	
O/N/A levels/diploma,	297	121.34 (1.78)		170.00 (2.54)	
University education,	217	121.79 (1.88)		171.91 (2.68)	
		Tortuosity A., x10^{-6}		**Tortuosity V., x10^{-6}**	
Ethnicity					
Chinese	450	108.20 (3.75)	<0.001	117.56 (4.07)	0.001
Malay	235	109.26 (3.92)		117.30 (4.26)	
Indians	139	120.62 (4.12)		125.85 (4.48)	
Household income					
SGD <1000 per mth	162	111.22 (3.92)	0.22	121.24 (4.26)	0.27
SGD 1000-2999 per mth	280	110.97 (3.96)		121.15 (4.30)	
SGD 3000-4999 per mth	209	115.30 (3.97)		120.51 (4.32)	
SGD ≥5000 per mth	173	113.27 (4.06)		118.05 (4.41)	
Maternal education					
Primary school,	38	107.67 (4.85)	0.05	124.38 (5.21)	0.31
Secondary school	232	111.44 (3.98)		117.95 (4.27)	
O/N/A levels/diploma,	297	113.73 (3.84)		120.75 (4.12)	
University education,	217	114.94 (4.00)		119.00 (4.29)	

		Retinal vascular parameters			
	N	**β (SE)**	**p trend**	**β (SE)**	**p trend**
		Branching angle A., D		**Branching angle V., D**	
Ethnicity					
Chinese	450	81.15 (2.04)	0.02	83.23 (1.92)	0.08
Malay	235	81.84 (2.13)		82.09 (2.01)	
Indians	139	84.08 (2.24)		85.38 (2.11)	
Household income					
SGD 0-1999 per month	162	81.90 (2.13)	0.44	83.03 (2.01)	0.60
SGD 2000-3999 per month	280	82.07 (2.15)		85.24 (2.03)	
SGD 4000-5999 per month	209	82.54 (2.16)		82.97 (2.04)	
>=SGD 6000 per month	173	82.93 (2.21)		83.03 (2.08)	
Maternal education					
Primary school,	38	78.58 (2.59)	0.04	82.92 (2.49)	0.82
Secondary school	232	81.92 (2.13)		83.66 (2.04)	
O/N/A levels/diploma,	297	83.19 (2.05)		83.55 (1.97)	
University education,	217	82.75 (2.13)		83.44 (2.05)	
		Fractal dimension A., Df, x10^{-3}		**Fractal dimension V., Df, x10^{-3}**	
Ethnicity					
Chinese	450	124.03 (1.00)	0.03	122.37 (0.91)	0.10
Malay	235	125.19 (1.04)		122.65 (0.95)	
Indians	139	125.40 (1.09)		121.41 (1.00)	
Household income					
SGD <1000 per mth	162	125.64 (1.04)	0.35	122.32 (0.95)	0.55
SGD 1000-2999 per mth	280	124.31 (1.05)		122.36 (9.58)	
SGD 3000-4999 per mth	209	124.72 (1.05)		121.78 (0.96)	
SGD ≥5000 per mth	173	124.81 (1.08)		122.10 (0.98)	
Maternal education					
Primary school,	38	125.19 (1.29)	0.92	121.63 (1.16)	0.47
Secondary school	232	125.06 (1.06)		122.48 (0.95)	
O/N/A levels/diploma,	297	124.94 (1.02)		122.10 (0.92)	
University education,	217	125.12 (1.06)		122.45 (0.95)	

Adjusted for age, ethnicity/household income/maternal education, hypertension, diabetes, smoking history, mean arterial blood pressure, body mass index and spherical equivalent.

Compared with Malay and Indians, Chinese pregnant women tended to have a significantly narrower retinal arteriolar caliber, smaller retinal arteriolar and venular tortuosity, smaller retrial arteriolar branching angle and smaller retinal arteriolar fractal dimension. Furthermore subjects who completed lower education tended to have smaller tortuosity and branching angles in retinal arterioles (**Table 60**).

3.4.2.2 Stratification and Interaction

A. STARS and STARS Family retinal study

Table 61. Significant Associations among Children Stratified by Age and Gender

		CRAE, µm			CRVE, µm		
	N	**β (SE)**	**p**	**p***	**β (SE)**	**p**	**p***
Systolic blood pressure (SBP), mm Hg							
Age, above 50th percentile	191	-2.67 (1.09)	0.02	0.06	2.01 (1.39)	0.15	0.08
Age, ≤ 50th percentile	191	-1.09 (1.24)	0.38		1.75 (1.72)	0.31	
Body mass index (BMI), kg/m^2							
Age, above 50th percentile	68	-0.67 (1.18)	0.57	0.78	4.94 (1.32)	<0.01	0.96
Age, ≤ 50th percentile	68	-2.21 (1.58)	0.17		0.39 (2.50)	0.88	
Axial length (AL), mm							
Age, above 50th percentile	233	-2.77 (2.23)	0.23	0.45	-1.05 (2.87)	0.72	0.73
Age, ≤ 50th percentile	236	-3.49 (2.16)	0.11		-8.34 (3.09)	<0.01	
Systolic blood pressure (SBP), mm Hg							
Boys	289	-2.31 (1.10)	0.04	0.09	1.32 (1.33)	0.32	0.20
Girls	297	-1.74 (1.05)	0.10		2.55 (1.52)	0.10	
Body mass index (BMI), kg/m^2							
Boys	62	1.49 (1.39)	0.29	0.60	1.77 (2.17)	0.42	0.13
Girls	74	-2.51 (1.17)	0.04		4.09 (1.38)	<0.01	
Axial length (AL), mm							
Boys	239	-5.84 (1.97)	<0.01	0.07	-8.26 (2.50)	<0.01	0.15
Girls	230	0.26 (2.46)	0.92		-1.94 (3.50)	0.58	

*p value for interaction of each pair

Age and gender were not effect modifiers in the relationships between SBP and retinal vascular caliber, nor in the relationship between BMI and retinal vascular caliber, nor in the relationship between AL and retinal vascular caliber (**Table 61**).

B. GUSTO retinal study

Table 62. Association between BP, BMI, PSQI and PSQI and Retinal Vascular Caliber among Pregnant Women Stratified by Age and Ethnicity

		CRAE, µm			CRVE, µm		
	N	β (SE)	p	p*	β (SE)	p	p*
Systolic blood pressure, mm Hg							
Age, above 50th percentile	268	-0.10 (0.04)	0.02	0.10	0.06 (0.06)	0.34	0.47
Age, ≤ 50th percentile	362	-0.10 (0.04)	0.02		0.01 (0.06)	0.99	
Pre-pregnancy BMI, kg/m^2							
Age, above 50th percentile	355	-1.19 (0.96)	0.22	0.13	0.61 (0.96)	0.53	0.15
Age, ≤ 50th percentile	439	-0.33 (0.64)	0.61		2.49 (1.21)	0.04	
EPDS, score							
Age, above 50th percentile	357	-0.01 (0.11)	0.99	0.28	-0.02 (0.16)	0.88	0.52
Age, ≤ 50th percentile	440	0.15 (0.10)	0.12		0.19 (0.13)	0.16	
PSQI, score							
Age, above 50th percentile	357	0.40 (0.20)	0.05	0.05	-0.01 (0.27)	0.97	0.60
Age, ≤ 50th percentile	440	0.21 (0.20)	0.28		0.01 (0.28)	0.97	
Systolic blood pressure, mm Hg							
Chinese	341	-0.08 (0.04)	0.03	0.10	-0.01 (0.05)	0.88	0.56
Malay	192	-0.15 (0.06)	0.01		0.07 (0.08)	0.42	
Indian	108	-0.10 (0.10)	0.32		0.06 (0.13)	0.66	
Pre-pregnancy BMI, kg/m^2							
Chinese	420	-1.04 (0.89)	0.25	0.48	1.96 (1.33)	0.14	0.36
Malay	224	-0.52 (0.72)	0.47		0.69 (0.98)	0.48	
Indian	139	0.43 (0.12)	<0.01		-1.07 (2.01)	0.61	
EPDS, score							
Chinese	421	0.76 (0.10)	0.46	0.86	-0.03 (0.15)	0.85	0.34
Malay	223	0.18 (0.14)	0.20		0.28 (0.20)	0.16	
Indian	153	0.07 (0.19)	0.71		0.18 (0.24)	0.46	
PSQI, score							
Chinese	421	0.46 (0.20)	0.02	0.56	-0.06 (0.30)	0.85	0.83
Malay	223	-0.08 (0.27)	0.76		0.68 (0.36)	0.07	
Indian	153	0.68 (0.33)	0.04		-0.76 (0.42)	0.08	

Table 63. Association between BP, BMI, PSQI and PSQI and Retinal Vascular Parameters among Pregnant Women Stratified by Age and Ethnicity

		Retinal vascular caliber					
	N	**β (SE)**	**p**	**p***	**β (SE)**	**p**	**p***
		Tortuosity A., x10^{-6}			**Tortuosity V., x10^{-6}**		
Pre-pregnancy BMI, kg/m^2							
Age, above 50th percentile	355	-1.71 (1.61)	0.29	0.21	3.73 (2.00)	0.07	0.05
Age, ≤ 50th percentile	439	-0.88 (2.14)	0.68		2.72 (1.71)	0.11	
Pre-pregnancy BMI, kg/m^2							
Chinese	420	-0.18 (2.50)	0.94	0.88	0.95 (2.24)	0.67	0.30
Malay	224	-1.35 (1.39)	0.34		3.13 (1.55)	0.05	
Indian	139	0.70 (3.69)	0.85		4.15 (5.11)	0.42	
		Branching angle A., D			**Branching angle V., D**		
Systolic blood pressure, mm Hg							
Age, above 50th percentile	268	-0.02 (0.05)	0.75	0.12	-0.01 (0.04)	0.78	0.39
Age, ≤ 50th percentile	362	-0.14 (0.05)	<0.01		-0.04 (0.04)	0.23	
Systolic blood pressure, mm Hg							
Chinese	341	-0.11 (0.05)	0.03	0.09	-0.03 (0.04)	0.34	0.26
Malay	192	-0.13 (0.08)	0.10		-0.05 (0.05)	0.36	
Indian	108	0.06 (0.10)	0.54		-0.01 (0.08)	0.93	
		Fractal Dimension A., x10^{-3}			**Fractal dimension V., x10^{-3}**		
Systolic blood pressure, mm Hg							
Age, above 50th percentile	268	-0.05 (0.03)	0.10	0.10	0.01 (0.03)	0.84	0.13
Age, ≤ 50th percentile	362	-0.01 (0.03)	0.57		0.04 (0.02)	0.12	
Systolic blood pressure, mm Hg							
Chinese	341	-0.50 (0.02)	0.04	0.08	-0.01 (0.02)	0.84	0.33
Malay	192	-0.03 (0.04)	0.52		0.05 (0.03)	0.15	
Indian	108	0.01 (0.05)	0.99		0.03 (0.05)	0.49	

*****p value for interaction of each pair

Neither age nor ethnicity modified any significant relationships between blood pressure/body mass index/EPDS/PSQI and retinal vascular parameters (**Table 62-63**).

3.4.2.3 Multiple Logistic Regression

Table 64. Multivariate Analysis of Association between Blood Pressure and Abnormal Retinal Vessel Signs

	N	Generalized retinal arteriolar narrowing Odds Ratio (95% CI)	Generalized retinal venular widening Odds Ratio (95% CI)
Systolic blood pressure			
Each 10 mmHg increase	665	1.03 (1.01, 1.06)	1.00 (0.97, 1.02)
Diastolic blood pressure			
Each 10 mmHg increase	665	1.05 (1.02, 1.09)	0.98 (0.95, 1.01)
Mean arterial blood pressure			
Each 10 mmHg increase	665	1.06 (1.03, 1.10)	0.98 (0.95, 1.01)
Preeclampsia risk group			
High risk, MABP≥90 mmHg	127	Reference	Reference
Low risk, MABP<90 mmHg	538	2.11 (1.00, 4.43)	1.54 (0.80, 2.98)

We also analyzed the association of blood pressure with generalized retinal arteriolar narrowing and retinal venular widening. Compared to the lowest quartile, pregnant women in the highest quartile of SBP were 2.40 times (odds ratio [OR] 2.40, 95% CI: 1.11, 5.22) more likely to have generalized retinal arteriolar narrowing. For DBP and MABP, the corresponding ORs were 2.8 (95% CI: 1.3, 6.1) and 2.5 (95% CI: 1.1, 5.6), and 2.3 (95% CI: 1.1, 5.0), respectively. Compared with participants in the low risk group (MABP <90 mmHg), participants in the high risk group (MABP ≥90 mmHg) were more than twice as likely (OR 2.1, 95% CI: 1.0, 4.4) to have generalized retinal arteriolar narrowing (**Tables 64**).

Table 65. Association between Retinal Vascular Parameters and Gestational Hypertension

		Gestational hypertension (n=20)	
		Model 1	**Model 2**
Retinal vascular parameter	**n**	**(OR, 95% CI)**	**(OR, 95% CI)**
CRAE, each 1.0 µm increase	665	0.94 (0.89, 0.99)	0.96 (0.90, 1.02)
Generalized retinal arteriolar narrowing			
No	531	Reference	Reference
Yes	134	4.27 (0.56, 32.47)	1.02 (0.90, 1.16)
CRAE, each 1.0 µm increase	665	0.97 (0.93, 1.01)	0.97 (0.93, 1.01)
Generalized retinal venular narrowing			
No	531	Reference	Reference
Yes	134	1.28 (0.36, 4.60)	1.57 (0.39, 6.40)
Retinal arteriolar tortuosity, x10-6			
Each 1.0 unit increase	665	0.99 (0.97, 1.02)	1.00 (0.97, 1.03)
Retinal venular tortuosity, x10-6			
Each 1.0 unit increase	665	1.01 (0.99, 1.03)	1.01 (0.98, 1.03)
Retinal arteriolar branching angle			
Each 1.0 unit increase	665	0.94 (0.89, 0.98)	0.94 (0.90, 0.99)
Retinal venular branching angle			
Each 1.0 unit increase	665	1.03 (0.98, 1.08)	1.02 (0.97, 1.08)
Retinal arteriolar fractal dimension, x10^{-3}			
Each 1.0 unit increase	665	0.94 (0.87, 1.02)	0.99 (0.91, 1.08)
Retinal venular fractal dimension, x10^{-3}			
Each 1.0 unit increase	665	1.03 (0.93, 1.14)	1.03 (0.92, 1.15)

Model 1, adjusted for age and ethnicity.

Model 2, adjusted for age, ethnicity, household income, hypertension history, diabetes history, systolic blood pressure, body mass index and Edinburgh Postnatal Depression Score.

Table 66. Association between Retinal Vascular Parameters and Pre-eclampsia

Retinal vascular parameter	n	Pre-eclampsia (n=16) Model 1 (OR, 95% CI)	Pre-eclampsia (n=16) Model 2 (OR, 95% CI)
CRAE, each 1.0 µm increase	665	0.95 (0.90, 1.01)	0.95 (0.88, 1.03)
Generalized retinal arteriolar narrowing			
No	531	Reference	Reference
Yes	134	1.60 (0.36, 7.19)	2.21 (0.02, 21.80)
CRAE, each 1.0 µm increase	665	0.99 (0.95, 1.03)	1.00 (0.95, 1.05)
Generalized retinal venular narrowing			
No	531	Reference	Reference
Yes	134	1.87 (0.41, 8.57)	1.90 (0.34, 10.51)
Retinal arteriolar tortuosity, x10-6			
Each 1.0 unit increase	665	0.98 (0.95, 1.02)	0.99 (0.95, 1.04)
Retinal venular tortuosity, x10-6			
Each 1.0 unit increase	665	0.99 (0.97, 1.02)	0.99 (0.96, 1.03)
Retinal arteriolar branching angle			
Each 1.0 unit increase	665	1.04 (0.98, 1.10)	1.06 (0.99, 1.13)
Retinal venular branching angle			
Each 1.0 unit increase	665	1.01 (0.95, 1.07)	1.00 (0.93, 1.07)
Retinal arteriolar fractal dimension, x10^{-3}			
Each 1.0 unit increase	665	0.92 (0.84, 1.01)	0.97 (0.87, 1.09)
Retinal venular fractal dimension, x10^{-3}			
Each 1.0 unit increase	665	1.01 (0.90, 1.13)	1.01 (0.88, 1.16)

Model 1, adjusted for age and ethnicity.

Model 2, adjusted for age, ethnicity, household income, hypertension history, diabetes history, systolic blood pressure, body mass index and Edinburgh Postnatal Depression Score.

Table 67. Association between Retinal Vascular Parameters and Gestational Hypertensive Disorders

		Gestational hypertensive disorders (n=36)	
		Model 1	**Model 2**
Retinal vascular parameter	**n**	**(OR, 95% CI)**	**(OR, 95% CI)**
CRAE, each 1.0 µm increase	665	0.94 (0.91, 0.98)	0.95 (0.90, 1.00)
Generalized retinal arteriolar narrowing			
No	531	Reference	Reference
Yes	134	2.54 (0.76, 8.48)	2.29 (0.49, 10.71)
CRAE, each 1.0 µm increase	665	0.98 (0.95, 1.01)	0.98 (0.95, 1.01)
Generalized retinal venular narrowing			
No	531	Reference	Reference
Yes	134	1.53 (0.57, 4.12)	1.67 (0.55, 5.09)
Retinal arteriolar tortuosity, x10-6			
Each 1.0 unit increase	665	0.99 (0.97, 1.01)	1.00 (0.97, 1.02)
Retinal venular tortuosity, x10-6			
Each 1.0 unit increase	665	1.00 (0.99, 1.02)	1.00 (0.98, 1.02)
Retinal arteriolar branching angle			
Each 1.0 unit increase	665	0.98 (0.94, 1.02)	0.98 (0.95, 1.02)
Retinal venular branching angle			
Each 1.0 unit increase	665	1.02 (0.98, 1.06)	1.01 (0.97, 1.06)
Retinal arteriolar fractal dimension, x10^{-3}			
Each 1.0 unit increase	665	0.93 (0.87, 0.99)	0.98 (0.91, 1.05)
Retinal venular fractal dimension, x10^{-3}			
Each 1.0 unit increase	665	1.02 (0.94, 1.10)	1.02 (0.93, 1.12)

Model 1, adjusted for age and ethnicity.

Model 2, adjusted for age, ethnicity, household income, hypertension history, diabetes history, systolic blood pressure, body mass index and Edinburgh Postnatal Depression Score.

Table 68. Association between Retinal Vascular Parameters and Hypertensive Disorders during Pregnancy (including chronic hypertension, gestational hypertension and pre-eclampsia)

		Hypertensive disorders during pregnancy (n=46)	
Retinal vascular parameter	**n**	**Model 1 (OR, 95% CI)**	**Model 2 (OR, 95% CI)**
CRAE, each 1.0 μm increase	665	0.95 (0.92, 0.99)	0.97 (0.92, 1.01)
Generalized retinal arteriolar narrowing			
No	531	Reference	Reference
Yes	134	2.64 (0.92, 7.57)	1.92 (0.50, 7.35)
CRAE, each 1.0 μm increase	665	0.98 (0.96, 1.01)	0.98 (0.95, 1.01)
Generalized retinal venular narrowing			
No	531	Reference	Reference
Yes	134	1.46 (0.63, 3.42)	1.84 (0.61, 5.53)
Retinal arteriolar tortuosity, x10-6			
Each 1.0 unit increase	665	0.99 (0.97, 1.01)	1.00 (0.97, 1.02)
Retinal venular tortuosity, x10-6			
Each 1.0 unit increase	665	1.00 (0.98, 1.02)	1.00 (0.99, 1.02)
Retinal arteriolar branching angle			
Each 1.0 unit increase	665	0.99 (0.95, 1.02)	0.99 (0.95. 1.03)
Retinal venular branching angle			
Each 1.0 unit increase	665	1.02 (0.98, 1.05)	1.02 (0.98, 1.06)
Retinal arteriolar fractal dimension, x10^{-3}			
Each 1.0 unit increase	665	0.92 (0.87, 0.98)	0.97 (0.91, 1.05)
Retinal venular fractal dimension, x10^{-3}			
Each 1.0 unit increase	665	1.01 (0.95, 1.09)	1.02 (0.93, 1.11)

Model 1, adjusted for age and ethnicity.

Model 2, adjusted for age, ethnicity, household income, diabetes history, systolic blood pressure, body mass index and Edinburgh Postnatal Depression Score.

Table 65-68 shows the association between retinal vascular parameters and different types of hypertensive condition during pregnancy by using multiple logistic

regression models. After adjusting for age, ethnicity, demographic status, medical history and systemic factors, retinal vascular parameters including retinal vascular caliber, retinal vascular tortuosity, retinal vascular branching angle and retinal vascular fractal dimension were not associated with occurrence of gestational hypertension, pre-eclampsia, gestational hypertensive disorders and chronic hypertension.

Furthermore, generalized retinal arteriolar narrowing was not associated with gestational hypertension (OR=1.02, 95% CI: 0.90, 1.16), pre-eclampsia (OR=2.21, 95% CI: 0.02, 21.80), gestational hypertensive disorders (OR=2.29, 95% CI: 0.49, 10.71) and hypertensive disorders during pregnancy (OR=1.92, 95% CI: 0.50, 7.35), respectively. Similarly, generalized venular widening was not associated with gestational hypertension (OR=1.57, 95% CI: 0.39, 6.40), pre-eclampsia (OR=1.90, 95% CI: 0.34, 10.51), gestational hypertensive disorders (OR=1.67, 95% CI: 0.55, 5.09), and hypertensive disorders during pregnancy (OR=1.84, 95% CI: 0.61, 5.53), respectively.

Table 69. Association between Retinal Vascular Parameters and Gestational Diabetes Mellitus (GDM) according to WHO Guideline

		Gestational diabetes mellitus (n=117)	
		Model 1	**Model 2**
Retinal vascular parameter	**n**	**(OR, 95% CI)**	**(OR, 95% CI)**
CRAE, each 1.0 µm increase		0.99 (0.97, 1.01)	1.00 (0.97, 1.04)
Generalized retinal arteriolar narrowing			
No		Reference	Reference
Yes		0.92 (0.56, 1.50)	1.26 (0.54, 2.98)
CRAE, each 1.0 µm increase		0.99 (0.98, 1.01)	1.00 (0.98, 1.03)
Generalized retinal venular narrowing			
No		Reference	Reference
Yes		1.11 (0.66, 1.88)	1.11 (0.48, 2.60)
Retinal arteriolar tortuosity, x10-6			
Each 1.0 unit increase		1.00 (0.99, 1.01)	1.02 (1.00, 1.04)
Retinal venular tortuosity, x10-6			
Each 1.0 unit increase		1.00 (0.99, 1.01)	1.00 (0.99, 1.02)
Retinal arteriolar branching angle			
Each 1.0 unit increase		1.02 (1.00, 1.05)	1.01 (0.97, 1.05)
Retinal venular branching angle			
Each 1.0 unit increase		0.99 (0.97, 1.02)	0.98 (0.95, 1.02)
Retinal arteriolar fractal dimension, x10^{-3}			
Each 1.0 unit increase		1.00 (0.99, 1.00)	1.00 (0.99, 1.00)
Retinal venular fractal dimension, x10^{-3}			
Each 1.0 unit increase		1.00 (0.99, 1.00)	1.00 (0.99, 1.01)

Model 1, adjusted for age and ethnicity.

Model 2, adjusted for age, ethnicity, household income, hypertension history, diabetes history, systolic blood pressure, pre-pregnancy body mass index and Pittsburgh Sleep Quality Index.

Table 69 shows the associations between retinal vascular parameters and gestational diabetes mellitus (GDM). In multiple logistic regression models, decrements in retinal vascular caliber, retinal vascular branching angle and retinal vascular fractal dimension were not associated with risk of GDM. However, after adjusting for age, ethnicity, household income, hypertension history, diabetes history, systolic blood pressure, pre-pregnancy BMI and PSQI, each unit increase in retinal arteriolar tortuosity was associated with 1.02 times (95% CI: 1.00, 1.04) the risk of GDM during the second trimester.

Chapter 4

4. Discussion

4.1 Summary of the Retinal Study in Singapore Children and Pregnant Women

In the population-based, cross-sectional study in 824 Singapore multi-ethnic pregnant women, blood pressure measurement, maternal obesity and antenatal mental health were associated with adverse retinal microvascular characteristics. This is the first comprehensive study to investigate the relationship between risk factors and changes in maternal retinal microcirculation during pregnancy. Our novel findings are: 1. Pregnant women with higher blood pressure (SBP, DPB and MABP) had an major impact on retinal arterioles, such as narrower retinal arteriolar caliber, smaller retinal arteriolar branching angle and smaller retinal arteriolar fractal dimension compared with those with lower blood pressure. Based on MABP reading at the second trimester, pregnant women classified into high risk group of getting incident preeclampsia had a range of relatively smaller retinal arteriolar parameters than those classified into low risk group. 2. Maternal obesity had major impacts both on retinal arterioles (e.g. retinal arteriolar narrowing) and retinal venules (e.g. wider and more tortuous retinal venules). 3. Maternal mental health worsening (e.g. depressive symptoms and poor sleep quality) was associated with retinal arteriolar widening. Our findings implicated that the impacts of maternal blood pressure, obesity and mental health status on microcirculation *in vivo* might provide helpful insight in understanding the pathphysiological mechanisms of developments of gestational complications (e.g. gestational hypertension, gestational diabetes, preeclampsia, etc.) induced by all these maternal risk factors mentioned above.

In our population-based, cross-sectional study in 586 Singapore Chinese children blood pressure measurements, body fatness indices and ocular biometric parameters were associated with a range of abnormal retinal vascular signs. This is the first study to investigate impacts of a series of systematic and optical risk factors on retinal microcirculation in very young children under the age of 6 years. The novel findings of these studies are: 1. Children with age, gender and height specific hypertension had significant narrower retinal arteriolar caliber compared with those normotensive children. 2. Children who were considered overweight and obesity with individual BMI and TSF above threshold had wider retinal venular caliber than those with normal weight. 3. Myopic children with longer axial length had narrower retinal arteriolar caliber and wider retinal venular caliber than emmetropic and hyperopic children. Our findings indicate that blood pressure, body fatness indices and ocular biometry have already predisposed early changes on retinal microcirculation among very young children.

4.2 Association between Blood Pressure and Retinal Microvasculature

The more important finding in our study is the association between blood pressure and retinal microvasculature among pregnant women and preschoolers. Abnormally elevated maternal blood pressure in the second or third trimester is associated with poor maternal and neonatal outcomes including preeclampsia,[219, 255, 266-268] preterm delivery[219] and low birth weigh,[215, 269] as blood pressure during uncomplicated pregnancy decreases physiologically towards mid-pregnancy and rises to preconception values at term[266, 270] in order to lower vascular resistance, enhance placental function and lead to improved pregnancy outcomes.[221] Similarly,

abnormally elevated blood pressure during childhood is associated with left ventricular hypertrophy,[208] cardiovascular risk factors,[209] and developments of hypertension [210, 211] and large vessel diseases such as carotid artery intima-media thickness in later life.[212] Nonetheless, whether blood pressure during pregnancy and childhood may predispose early impacts on vascular pathological changes and lead to a series of future adverse outcomes remains uncertain. In part, this is because the microcirculation *in vivo* is difficult to examine in pregnant women and young children.

Due to the fact that blood pressure is one of the most important systematic factors and it links up with different conditions and diseases across all populations, this relationship between blood pressure and retinal microcirculation among pregnant women and very young children helps to fill up the gaps in recent studies and shed lights on relevant pathophysiological mechanisms for both unique populations.

In pregnant women, every 10 mmHg increase in mean arterial blood pressure was associated with a 1.9 μm ($p<0.001$) reduction in retinal arteriolar caliber, a 0.9° ($p=0.05$) reduction in retinal arteriolar branching angle, and a 0.07 ($p<0.01$) reduction in retinal arteriolar fractal dimension, respectively. Participants classified into high-risk group in developing pre-eclampsia (mean arterial blood pressure ≥ 90 mmHg) was twice as likely (Odds Ratio: 2.1, 95% confidence interval: 1.0, 4.4) to have generalized retinal arteriolar narrowing compared with those classified into low-risk group (mean arterial blood pressure < 90 mmHg).

In preschoolers, each 10-mmHg increase in SBP was associated with an approximate 2.00 μm narrower retinal arteriolar caliber and a 2.51 μm wider retinal

venular caliber. Children classified as having age, gender and height specific hypertension (n=70) had approximately 4 µm narrower retinal arteriolar caliber (p=0.02 for trend) than children who were classified as normotensive (n= 315) in the same multivariate analysis.

Our major finding which showed that higher systolic blood pressure was related to narrower retinal arteriolar caliber among pregnant women and young children is comparable with other studies in older children[62, 73] and in adults.[8, 9, 17, 28, 29, 59, 83, 271, 272] 20 years ago, Skalina *et al* and Daniel *et al* observed newborn infants, children and adolescents with essential hypertension, and reported both decreased Arteriole-to-Venule Ratio (AVR)[273] and retinal arteriolar narrowing[274] by using qualitative methods. In SCES, each 10 mm Hg increase in SBP was independently associated with a 2.08-µm decrease ($p<0.001$) and a 1.46-µm decrease ($p<0.0001$) in retinal arteriolar caliber among 1572 Caucasian children aged 6-8 years [62] and among 2272 Caucasian pre-adolescents with mean age 12.7 years[73], respectively. In SCORM, each 10 mm Hg increase in SBP was independently associated with a 1.43-µm decrease ($p<0.001$) in retinal arteriolar caliber among 380 Singapore children aged 6 to 9 years [62]. Interestingly, there was a significant blood pressure threshold effect, which was defined as hypertension by referring to age-, gender- and height- specific systolic blood pressure and/or diastolic blood pressure $\geq 95^{th}$ percentiles,[248, 249] on retinal arteriolar narrowing among 70 Singapore Chinese children in our study. This finding was comparable to the SCES on pre-adolescents[73] and other adulthood studies.[8, 9, 28, 29, 83, 271, 272]

Furthermore, recent studies have shown that examining the geometric branching pattern of the retinal vascular tree, which may capture the "optimal state" of the retinal microcirculation, are also influenced by blood pressure. Narrower retinal arteriolar branching angle and reduced fractal dimension has been reported to be associated with higher levels of blood pressure,[5, 11, 35] and suboptimal fractal dimension has also been linked to acute lacunar stroke,[12] chronic kidney disease,[202] and coronary heart disease mortality.[41] Consistently, our study shows that elevated blood pressure is possibly associated with a range of adverse retinal arteriolar parameters, including narrower retinal arteriolar caliber, smaller retinal arteriolar branching angle and smaller retinal arteriolar fractal dimension. The findings provide evidence of an impact of blood pressure on the microcirculation during pregnancy and further insights into physiological and hemodynamic changes during pregnancy.

The retinal vasculature is a unique "window" for monitoring the microcirculative system, and blood pressure is regulated through complex physiological mechanisms such as endothelial function, arterial compliance, sympathetic nervous system, hypothalamic pituitary adrenal (HPA) axis and rennin-angiotensin system.[207] The pathophysiological changes in retina in response to blood pressure elevation are widely hypothesized as hypertensive retinopathy.[1, 8] Moreover, it has been reported that smaller arteriolar caliber, and wider retinal venular caliber are related to future clinical stroke[275, 276] and coronary heart disease morbidity and mortality in adults;[178, 226, 277, 278] suggesting that retinal vascular imaging might permit physicians to be aware of subclinical hypertension in healthy adult population, or to optimize management such as appropriate preventative strategies for stroke or cardiovascular

disease in adult patients.[279] Recent works in populations without diabetes suggest that retinal vascular caliber measurements provide slightly superior coronary heart disease risk prediction compared to the use of traditional risk factors such as blood pressure alone.[176] McGeechan et al reported that an area under the receiver operator characteristic curve increased from 0.695 to 0.706 (1.7% increase) with the addition of retinal vascular caliber to the Framingham risk model in women participants.[38] In this sense, blood pressure measurement might not be an adequate way to predict future events in the clinical setting alone, and vigorous evidence shows that there is a more predictive usage of retinal vascular caliber measurement.

The maternal cardiovascular system undergoes profound changes during pregnancy, as well as in women with complicated pregnancies. In our study, elevated blood pressure was associated with retinal arteriolar narrowing, narrower retinal arteriolar branching angle and smaller retinal arteriolar fractal dimension. These changes may reflect a range of possible mechanisms including vasospasm, decrease in nitride-oxide (NO) release,[3] endothelial dysfunction,[11] impairment of perfusion or oxygenation in retinal microvasculature.[280] Because the human circulatory system is believed to conform an 'optimum design' based on Murray's principle of minimum work,[281] deviations or alterations from the optimal retinal vascular architecture are associated with impaired microcirculatory transport and reduced efficiency,[33, 34, 281] and can be a marker of increase cardiovascular risk and poorer pregnancy outcomes. Further studies are required to explore the hemodynamics of the retinal microcirculation in response to elevated blood pressure.

Retrospective studies have shown that childhood blood pressure is independently associated with hypertension,[282] the development of atherosclerosis in larger arteries such as the carotid, aorta or femoral vessels,[212, 283] left ventricular hypertrophy[208] and cardiovascular mortality in adulthood.[211] According to this hemodynamic concept, vasospasm provoked by both elevated blood pressure and an increase in vasomotor tone due to local autoregulation may lead to a consequent elevation in capillary pressure and flow.[3, 284] Since such hemodynamic-induced pathophysiological vascular changes are likely to represent a continuous process, it will be valuable to continue the follow up of very young children with these signs, to assess their course and association with blood pressure in the longer term, independent of other predictors like BMI.

It is not known at present whether the documentation of retinal arteriolar narrowing during uncomplicated pregnancy and early childhood would predict the incidence of gestational outcomes (e.g. pre-eclampsia and fetal growth) upon delivery and incidence of vascular complications (e.g. hypertension and cardiovascular disease) upon adulthood, respectively. Further longitudinal studies which aim to assess the existence and magnitude of independent associations between blood pressure, retinal vasculature and vascular complications in both pregnant women and young children could advance our knowledge considerably.

4.3 Association between Obesity, Body Fatness Indices and Retinal Vasculature

Substantial evidence has shown that maternal obesity is a risk factor for gestational complications (e.g. hypertension, diabetes and preeclampsia) and it will increase postpartum rates of hyperlipidemia and cardiovascular diseases[231-234]. Pre-

pregnancy body mass index (BMI) has been traditionally used for determining maternal obesity status, while pregnancy weight gain and BMI have been correlated with hemodynamic and metabolic changes including physiological increases in fat, uterine, breast tissue, extracellular fluid and fetal and placental growth[285, 286]. The underlying mechanisms and pathways linking maternal obesity with gestational and postpartum vascular complications are poorly defined and are complex[232, 287].

As for childhood overweight and obesity, the worldwide prevalence has increased greatly in most industrialized countries and several low-income countries during the past two to three decades[288]. Numerous epidemiological studies have shown that childhood overweight/obesity can adversely affect almost every organ system and cause relevant disorders such as asthma[225], pubertal advancement[223], glomerulopathy[224] and lower-limb malalignment[289], and is associated with increased risk of many chronic diseases in adulthood like type 2 diabetes, hypertension, cardiovascular disease and gastro-oesophageal reflux[222].

It has been hypothesized that small vessel endothelial dysfunction may partly underlie the link between obesity and cardiovascular risks, but the contribution and exact role of the microcirculation during pregnancy and childhood is unclear due to difficulties in its assessment[235]. Retinal microcirculation again may provide valuable information in deducing the pathophysiological mechanisms of developments of cardiovascular risks imposed by maternal obesity and childhood obesity. Therefore, this study brings out the second important message, which is that maternal obesity, childhood overweight and obesity, body fatness indices (BMI and triceps skinfold) were all significantly associated with changes in retinal vasculature.

In pregnant women, each SD increase of 26-week pregnancy BMI (4.57 kg/m^2) was associated with narrower retinal arteriolar caliber (by 1.58 µm, p<0.001), wider venular caliber (by 1.28 µm, p=0.02) and increased retinal venular tortuosity (p=0.01). Compared with mothers with normal weight, obese mothers (pre-pregnancy BMI>30.0 kg/m^2) had narrower retinal arteriolar caliber (118.81 vs. 123.38 µm, p<0.001), wider retinal venular caliber (175.81 vs. 173.01 µm; p<0.01) and increased retinal venular tortuosity (129.92 vs. 121.49 x10^{-6}; p<0.01). Pregnant women whose BMI-specific weight gain from pre-pregnancy to 26 weeks gestation above Institute of Medicine recommendations had narrower retinal arteriolar caliber (120.68 µm) than women with ideal (121.91 µm) and less than ideal weight gain (123.17), respectively (p_{trend}=0.05).

In children, each SD increase in BMI (3.52 kg/m^2) and triceps skinfold (4.49 mm) was associated with a 3.40-µm (p=0.005) and a 2.94-µm (p=0.012) widening in retinal venular calibre, respectively. Compared with children with BMI and triceps skinfold below threshold, children with BMI and triceps skinfold above threshold had a 9.33-µm (p=0.021) and a 10.21-µm (p=0.001) increase in retinal venular calibre, respectively.

Some epidemiological studies have reported that indices of adiposity such as BMI, waist-to-hip ratio and triceps skinfold are associated with retinal arteriolar narrowing and/or retinal venular widening in both adults and children[6, 9, 106, 290, 291]. Wong *et al* studied a multi-ethnic population of 5979 Americans aged 45 to 84 years and reported that each SD increase (5.4 kg/m^2) in BMI was associated with retinal venular widening (by 2.21µm, p<0.001)[9]. In a study of 2353 Australian adolescents with a

mean age of 12.7 years, Gopinath *et al* found that obese adolescents had a narrower retinal arteriolar caliber (by 2.8µm, p=0.01) and a wider retinal venular caliber (by 4.5µm, p=0.01) than adolescents with normal weight[6]. It has been speculated that the mechanisms underlying such associations might include oxidative stress, reduction in nitric-oxide (NO) production and chronic inflammation[6, 106, 290, 291].

Consistent with previous studies in non-pregnant adults and adolescents[6, 106, 290, 291], we found associations between either maternal pre-pregnancy or childhood BMI and retinal vascular changes. Mothers with higher pre-pregnancy or pregnancy BMI were more likely to have retinal arteriolar narrowing and/or retinal venular widening and/or more tortuous retinal venules. Children with higher BMI and higher triceps skinfold were more likely to have retinal venular widening. Based on WHO or CDC cut-off for maternal obesity or childhood overweight and obesity, maternal obesity and childhood overweight/obesity were both associated with retinal venular widening.

Even though maternal obesity is associated with a series of adverse maternal disorders including gestational hypertension, gestational diabetes and pre-eclampsia together with higher rates of cesarean delivery and postpartum hyperlipidemia and cardiovascular diseases[9, 106, 290, 292, 293], data on the quality of the microcirculation among pregnant women is largely lacking. BMI has been used widely to screen for overweight and obesity among adults; but as a measure of weight relative to height, it is only a proxy used to estimate body fatness[250, 294]. Therefore, we also studied the association between TSF and retinal vascular caliber and examined the age- and gender-specific TSF threshold in our study. To our knowledge, there is no previous comparable study which has investigated the relationship between TSF and retinal

vascular calibre both in children and adults. We found that higher quartile of TSF was associated with wider retinal venular caliber which was consistent from unadjusted model to multivariate model and showed more sensitivity than the association of BMI and retinal venular caliber by comparing the p-trend value in multivariable model (0.008 vs. 0.033) (Table 2). In the same model after controlling age, gender, father's education, SBP, axial length and fellow vessel, each SD increase of TSF (4.49 mm) was associated with a 2.94-μm (95% CI: 0.65, 5.63; p=0.012) widening in retinal venular caliber. Children with TSF above threshold had a 10.21-μm widening in retinal venular caliber than those with TSF below the threshold (p=0.001). Again, this significance of association in TSF threshold and retinal venular caliber was more sensitive than that in BMI threshold and retinal venular caliber (0.001 vs. 0.021).

Maternal cardiovascular hemodynamic adaptations to pregnancy include increased cardiac output, heart rate and stroke volume[270]. Some studies have suggested that women with higher BMI are subjected to a greater risk of adverse maternal hemodynamic changes compared with their leaner counterparts, including higher arterial blood pressure, hemoconcentration, impaired blood flow and altered cardiac function[295, 296] and put them at risk of hypertensive disorders[287, 297-303] and venous thromboembolism[304]. Maternal obesity and excessive gestational weight gain is independently associated with increased insulin-resistance and has a greater risk of gettomg gestational diabetes, and microvascular dysfunction is implicated in these disorders. Our study of pregnancy associated changes in the retinal microvasculature may provide a valuable approach to evaluating the pathogenesis of these pregnancy associated diseases [17, 305, 306].

Interestingly, Ko et al[307] and He et al[308] both suggested that an increase of hypertension, type 2 diabetes and cholesterolaemia in Asian Chinese had developed for a shorter time, in a younger age group, and in people with lower BMI ($\geq$25 kg/m^2) when compared with Caucasians from Europe and the United States. This pronounced differences in Asian Chinese included the high proportion of body fat and prominent abdominal obesity compared with those of European origin with similar BMI values[308, 309]. This characteristic implies that Asian Chinese might have a higher predisposition to insulin resistance at a lesser degree of obesity than people of European descent[308, 309], which has the pronounced dysfunction in early insulin secretion[310, 311]. Thus, those measurements for evaluating body fatness like TSF might be more appropriate indices of overweight or obesity for Asian Chinese. In our study, we found that TSF was associated with retinal vascular widening among Singapore Chinese children aged 6-16 years old. This helps "track" the "unique" obesity predisposition in Asian Chinese by evaluating body fat distribution early in young Asian Chinese children, and help provide new insights into the physiological basis of bodyweight regulation as well.

It is hypothesized that obese subjects have increased markers of endothelial activation and chronic vascular inflammation, Kuo et al suggested that intravitreous administration of low doses of an *Escherichia coli* endotoxin to humans led to an increase in white blood cell count and retinal venular dilation[312]. And Tamai et al reported that administration of lipid hydroperoxide into the vitreous humour of rats increased the number of leukocytes in the retinal microvasculature and the size of the retinal venular caliber, but not arteriolar caliber[198]. Furthermore, there are other

changes seen in obese subjects, such as increased blood volume[200] and hyperleptinemia[199], which might bring up oxidative stress and NO dysregulation[313] and later trigger retinal venular dilation. Based on these assumptions, it might be implied from our STARS Family study that higher TSF and BMI level could also be linked to increased white blood cell or higher leptin level, which are related to retinal venular dilation.

Findings on body fatness indices (TSF and BMI) and retinal venular widening in children might help to provide further evidence of the relationship between childhood overweight and obesity and systemic diseases later in life. Studies performed in the United States and Europe have found that about one-third of overweight and obese children remained overweight and obese as adults[314-316], which is well established to be associated with the metabolic syndrome[317]. Several studies have reported that childhood obesity is associated with adverse influence on almost every organ system and ill consequences later in life, including hypertension, dyslipidaemia, insulin resistance, fatty liver disease, or even psychosocial complications, and cardiovascular disease[222, 318, 319]. The association between obesity indices (TSF and BMI) and retinal venular calibre in children can further enlighten us on the pathophysiological mechanisms of how childhood overweight and obesity leads to various complications in adulthood such as hypertension, diabetes, cardiovascular morbidity and mortality.

4.4 Association between Ocular Biometric Parameters and Retinal Vascular Caliber among Children

The socioeconomic cost associated with myopia is significant since it affects an individual for a lifetime.[1] This burden is particularly large among Asian Chinese with high myopic prevalence such as seen in Singapore,[236] Hong Kong[320] and Taiwan.[321] It has been widely studied that pathologic myopia is associated with potentially blinding complications such as retinal detachment, choroidal neovascularization and macular holes,[322-324] incident glaucoma and incident cataract.[325, 326] By using Doppler ultrasonography, reduction of retinal blood flow was indirectly reported in highly myopic eyes (≥-6.0D)[327] or even more degenerative myopic eyes (≥-8.0D),[328] myopic eyes with open-angle glaucoma[329] and myopic eyes with choroidal neovascularization.[330] Studying microvascular changes through examining retinal vascular caliber on myopic eyes may lead to better understanding of the pathophysiology of myopia.

Among all ocular biometric parameters in our study, longer axial length was associated with narrowing in both retinal arteriolar and venular caliber. Larger corneal curvature was only associated with narrowing in retinal venular caliber, and anterior chamber depth was not associated with either retinal arteriolar or venular caliber.

There have been only a few studies that have reported the relationship either between axial length and retinal vascular caliber or between refractive error and retinal vascular caliber, mainly in adults.[10, 95, 331, 332] Patton *et al.* reported the negative association between axial length and retinal venular caliber by using Pearson's coefficient correlation (R=-0.28; p=0.04).[332] In Singapore Malay Eye Study (SiMES) conducted in adults aged 40-80 years, Lim *et al.* reported that per 1.0mm increase in

axial length, there was an associated 3.25μm and a 3.20μm narrowing in retinal arteriolar and venular caliber, respectively[331]. Similar to axial length, SE was suggested to be positively related to wider retinal arteriolar and venular caliber in the Beaver Dam Eye Study (BDES)[10] and SiMES.[331] BDES found that each 1.0 diopter decrease in SE was significantly associated with a 2.8μm decrease and 3.3μm decrease in retinal arteriolar and venular caliber, respectively.[10] SiMES found a much smaller decrease in both retinal arteriolar caliber and venular caliber as 0.46μm and 0.42μm, respectively.[331] If refraction category was taken into account with the trend changed from hyperopia to myopia, the Blue Mountain Study reported a decreasing trend in both retinal arteriolar caliber (204.7 μm vs. 162.5μm; $p<0.001$) and retinal venular caliber (238.9μm vs. 195.9μm; $p<0.001$),[95] while the SiMES study only reported a decreasing trend in retinal venular caliber from hyperopia to myopia (204.35μm vs. 202.08μm; $p=0.02$).[331] For children, there has been only one study on axial length with retinal vascular caliber among children aged 7-9 years, and the findings were similar to adult study mentioned above. [14] The Singapore Cohort Study of the Risk Factors for Myopia (SCORM) reported per SD (1.02 mm) increase in axial length was statistically associated with a 3.18μm and a 4.62μm decrease in retinal arteriolar and venular caliber, respectively.[88]

It has been widely suggested that abnormal retinal vascular signs are related to systemic outcomes such as hypertension and diabetes.[21-24] Up till now it has not been applied to ocular outcome yet.[13, 14, 16, 17] However, the application of retinal fellow vessel model in ocular outcomes should be explored more for practical statistical analysis. In order to be able to relate our findings with those reported in previous

studies on similar topic,[13, 14, 16, 17] we prefer to take the adjustment without retinal fellow vessel as our ultimate analysis.

Increased axial elongation in myopes may lead to mechanical stretching and thinning of the choroid and retinal pigment epithelium with concomitant vascular and degenerative changes.[333] Regarding the possible concomitant vascular changes on retinal microcirculation, especially speculated less ocular blood flow, studies have shown some direct and indirect evidence.[327, 328, 330, 331, 334, 335] By using Doppler ultrasonography or Doppler velocimetry, reduction of retinal blood flow velocity or reduced retinal blood flow was reported in high myopic eyes (>-8.0D),[327, 328] myopic eyes with open-angle glaucoma[329] and myopic eyes with choroidal neovascularization.[330] By using retinal photography, retinal vascular caliber and retinal vessel geometry were all decreased in myopic eyes, which implied a decreased retinal microcirculation in myopic subjects.[331] By using three-dimensional magnetic resonance imaging (MRI), axial globe enlargement to achieve a prolate shape in young children's myopic eyes was seen, which implied that a stretched eye ball and increased axial elongation in myopes probably led to mechanical stretching and thinning of the choroid and retinal pigment epithelium with concomitant vascular and degenerative changes.[335, 336] If a given eye begins development with a set complement of retinal vasculature, it is likely that a pathological increase in ocular dimensions could cause stretching and elongation of the retinal vessels, leading to reduction of the retinal vessel width.

The clinical implications of our study might lie in two parts. Firstly, Saw *et al.* suggested that patients with myopia, especially high myopia, may have higher risks of

cataract, glaucoma, and chorioretinal abnormalities such as retinal detachment and optic disc abnormalities.[336] Therefore, the relationship between axial length and retinal vascular caliber can shed light on the underlying pathophysiological mechanisms on how myopic subjects progress to develop pathologic implications. Secondly, in a recent study by Lim *et al.*, myopic refraction and longer axial length were associated with a lower risk of diabetic retinopathy, particularly vision-threatening retinopathy[337]. It was hypothesized that chorioretinal thinning among high myopic individuals may be protective, both by reducing the metabolic demands of the retina and by facilitating diffusion of oxygen through the retina. Therefore, the reduced metabolic demands of the retina might be directly caused by the reduced blood flow established in this study and the previous studies.

Cavallini *et al.* and Shimmyo *et al.* found a highly significant correlation between corneal curvature and central corneal thickness.[338, 339] And thinner central corneal thickness (CCT) was reported to be associated with retinal arteriolar narrowing in SCORM study.[91] Furthermore, intraocular pressure (IOP) measurements by applanation tonometry are affected by central corneal thickness, corneal curvature and axial length.[340] Interestingly, we found a very strong correlation between retinal venular caliber and corneal curvature across both models. Based on the previous study findings, a few possible mechanisms might lead larger corneal curvature to retinal vascular caliber changes. Firstly, the corneal curvature might share the same viscoelastic properties of lamina cribrosa as CCT does.[341] Larger corneal curvature might share the same mechanism as thinner CCT, which was associated with a thinner lamina cribrosa and the reduced mechanical support for blood vessel passing

through would in turn lead the compression of retinal vessel walls.[341] Secondly, larger corneal curvature might imply a latent higher IOP, which had a direct impact on retinal vascular narrowing.[342, 343] However, our study only found the association between larger corneal curvature and retinal venular narrowing, which might not be fully explained by these two hypothized theories mentioned above. Due to lack of literature support, it is unclear how corneal curvature was related to retinal venular caliber yet not to retinal arteriolar caliber. Whether this association is biologically proven or just statistically proven still needs more studies to identify.

4.5 Association between Antenatal Mental Health and Retinal Vascular Parameters among Pregnant Women

Depression, anxiety and poor sleep have been well established to be associated with cardiovascular diseases both in general population and clinical patients with obesity or coronary heart disease.[238-242] Growing evidence also shows that depression and anxiety during pregnancy is related to adverse maternal outcome such as preeclampsia[243] by possibly sharing similar pathophysiology with cardiovascular disease (CVD).[244] Since antenatal depression is commonly accompanied by anxiety and poor sleep[245, 246] and it affects up to 12.8% of pregnant women[247] in Caucasian population and 12.2% in Singapore population[237] during the second trimester, antenatal mental health has become an important public health issue for its influence on further vascular complications. Therefore, it is necessary to study the precise pathophysiological mechanisms to enlighten the association of antenatal depression, anxiety and poor sleep with hemodynamic circulation.

In this study, we reported that antenatal depressive symptoms and poor sleep quality were significantly associated with wider retinal arteriolar caliber and possibly associated with wider retinal venular caliber in Asian pregnant women. Antenatal anxious symptoms were not associated with retinal vascular caliber.

Thus far, several previous studies have reported inconsistent results. Nguyen *et al.* found a trend of widening retinal arteriolar caliber across the control group, the group of type 2 diabetic patients without depression and the group of type 2 diabetic patients with major depression (p-trend=0.02),[344] while Cheung *et al.* reported that "vital exhaustion", which was measured by Maastricht questionnaire to capture symptoms of unusual fatigue and feelings of rejection, was associated with generalized retinal venular widening.[114] Two other cross-sectional studies also reported an association between retinopathy signs with depressive moods.[46, 345] However, prospective data from the Rotterdam Study consisting of 3,605 participants who were followed for an average 9.0 years, reported that retinal vascular calibers were associated neither with incident depressive symptoms nor depressive syndrome [346].

In contrast to previous studies, the present study focused on antenatal depression, which refers to both major and minor episodes during pregnancy.[347] Antenatal depression is reported to be the most common mental condition during the second trimester of the pregnancy.[3] Several mechanisms may hypothetically underlie the associations between mental problems during pregnancy and wider retinal arteriolar caliber including inflammatory response and endothelial dysfunction. Firstly, Gold *et al* showed that hypercortisolism in patients with major depression could contribute to

increases in visceral fat mass, portal and peripheral free fatty acids, which further promotes endothelial inflammation in terms of higher level of tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), and C-reactive protein (CRP).[245] In the early phase of an inflammatory response, hyperemia is often noted and it probably reflects an initial reaction of arterioles.[348] Endothelial cells may be the main factor contributing to the early dilatory response of arterioles to inflammation, by means of producing more nitric oxide (NO) to further relax vascular smooth muscle.[348] Secondly, impaired brachial artery flow-mediated dilation (FMD) and depletion of circulating $CD34/KDR^{+}$ endothelial progenitor cells (EPC) has also been found to be associated with higher depression and anxiety scores in a recent study, examining the link between vascular disease and depression.[349] Lastly, estrogen-mediated endothelial NO synthase (eNOS) upregulation modulates eNOS expression in the fetal pulmonary endothelium, optimizing the capacity for NO-mediated pulmonary vasodilation at birth.[350] It might imply some eNOS-mediated effects on maternal vasodilation due to the rapid increase of gestational estrogen. Unfortunately, we don't have non-pregnant Singapore women as a control group to provide the baseline association between depression and retinal vascular caliber. Therefore, we won't be able to further explain whether this association between antenatal depressive symptoms and retinal arteriolar widening is specific for pregnant women.

Although anxiety is often a comorbid disorder with depression during pregnancy, we did not observe any significant associations between anxiety and retinal vascular caliber. This is in agreement with Chen *et al.*[349] in Hong Kong Chinese yet in disagreement with Jensen *et al.* in multi-ethnic Americans.[46] The possible explanation

for this might be due to differences in the nature of studied population such as age, ethnicities, duration of anxiety and specific hormone level *in vivo*. Thus, it requires further studies to investigate the relationship between antenatal anxiety and retinal microcirculation.

As poor sleep is prevalent in patients with major depression with up to 90% of patients reporting frequent insomnia symptoms,[241] it is not surprising to find that sleep was also associated with changes in retinal arteriolar caliber in our study. It has been shown that sleep is crucial for the maintenance and restoration of homeostasis through the regulation of energy, repair and infection control and is important for neuronal activity.[240] Consequently, deprivation of sleep or sleep disorders may be associated with CVD disease.[351, 352] Similarly to antenatal depression, sleep quality may affect susceptibility to infection with increased levels of high-sensitivity C-reactive protein (CRP), TNF-α, IL-6 and inflammatory cytokines.[353, 354] Our study showed that poorer sleep quality was indeed associated with wider retinal arteriolar caliber.

Compared with healthy subjects, a few functional neuroimaging studies by using computed tomography in interested cerebral regions reported that increased cerebral blood flow and elevated cerebral glucose metabolism were present in adults with depression.[355-357] Since retinal and cerebral 14 vessels shared similar anatomic, embryologic and functional features, retinal vascular abnormalities were shown to be closely correlated with cerebral circulation.[3] Our finding that pregnant women with antenatal depressive symptoms and poor sleep quality had greater retinal arteriolar vasodilation indirectly implied a possible increased cerebral blood flow in adults with

depression. Thus, reflected from our study, the retinal vascular imaging may also shed light on the clinical practice on investigating the correlation between mental health and cerebral functioning.

4.6 Associations between Gender and Retinal Vascular Caliber in Children

Even though there are plenty of studies establishing the association between age and retinal vascular caliber; there is still a lack of evidence showing a relationship between gender and retinal vascular parameters in general population. In our Singapore Chinese children, boys had an average 4.87 μm narrower retinal arteriolar caliber than girls in the same study population. This result was consistent with the Blue Mountain Eye Study, which reported higher retinal arteriolar caliber and AVR in women than in men[39].

A possible explanation of such gender difference is the vasodilating effect of estrogen. Estrogen, through a receptor-mediated pathway, may upregulate endothelial expression of the nitric oxide synthase gene, leading to increased nitric oxide production and resultant arteriolar dilatation.[358]

4.7 Associations between Ethnicity and Retinal Vascular Parameters in Pregnant Women

The study on relationship of ethnicity and retinal vascular parameters has been quite fundamentally studied, since some cardiovascular risk factors varied in different ethnic groups. In SCES, East Asian had a 11.1 μm wider retinal venular caliber than European Caucasian.[44] In MESA, Hispanic and Black participants had a wider retinal arteriolar and venular caliber than Chinese and White participants.[9] Similarly in SCORM children study, Cheung et al. found there is an increasing trend in retinal

venular caliber across Chinese children, Indian children and Malay children (p trend=0.02).[45] Mahal et al. studied 51 subjects with type 2 diabetes aged 40-65 years and found African-Caribbean had wider retinal arteriolar caliber (82 vs. 76 μm, p=0.03) than European.[21]

In our study among pregnant women, Chinese had the smallest retinal arteriolar caliber, the smallest retinal arteriolar and venular tortuosity, the smallest retinal arteriolar branching angle and the smallest retinal arteriolar fractal dimension among three ethnic groups. The associations between ethnicity and retinal vascular parameters indicate a reflection of relationship between genetic variance and retinal vascular caliber.

4.8 Associations between Demographic Status and Life Style and Retinal Vascular Parameters in Children and Pregnant Women

Even though the relationship between life style (cigarette smoking and alcohol drinking), lower education level and lower socio-economic status and retinal vascular caliber were repeatedly reported by a few large population-based and cross-sectional studies on muddle-to-old age subjects,[8, 9, 28, 37, 47, 48], there is no such association found in our children and pregnant women retinal study.

The negative finding in children was hypothesized that these indirect influences imposed by parental socio-economic status and life style might be attenuated while passing onto the second generation. The negative finding in young pregnant women (mean age=30.51 years) might be due to the relatively shorter exposure of smoking, which is unlike those positive findings reported in middle-to-old age participants with

longer cigarette smoking history and higher risk in developing cardiovascular diseases.[8, 10, 11, 137-139]

4.9 Associations between Birth Parameters and Retinal Vascular Caliber in Children

A large body of literature shows that susceptibility to cardiovascular disease may have etiological origins in utero and in infancy.[144-146] In epidemiological data reported by quite a few population-based studies, it was suggested that poorer early life growth such as lower birth weight and smaller head circumference was associated with higher blood pressure in childhood[146-148] and risk of cardiovascular disease in adulthood.[149] Based on these findings, researchers have hypothesized that early growth may affect small vessel structure and function.

It has been repeatedly reported that smaller birth parameters were associated with retinal arteriolar narrowing, smaller fractal dimension or retinal venular widening both in children and adults.[150-154] Each SD decrease in birth weight was associated with a 1.28 μm narrowing in retinal arteriolar caliber and a 1.49 μm in retinal venular caliber.[151-154] In SCES, Mitchell found a 3.73 μm narrower retinal arteriolar caliber among children with low birth weight (<2500 g) than those with normal birth weight.[151] Similar magnitude was found in retinal arteriolar narrowing with each SD decrease in other birth parameters such as birth length and head circumference.[150, 151, 153] For gestational age, premature babies had a 3.43 μm narrower retinal arteriolar caliber than full-term babies.[151] The changes in retinal arteriolar caliber might be the early predisposition of vascular malformation which is maladaptive in later life.

It has been hypothesized that the abnormal retinal vascular pattern in preterm children reflects more widely spread vascular damage.[359] Some of the structural alterations studied (e.g., narrower arteriolar angle) have been linked with impaired mechanical efficiency of the general vascular network in the body, leading to greater workload on the cardiovascular system and subsequent increase in risk of cardiovascular disease development.[360] The collective data from these studies underscore the importance of assessing architectural changes in the retinal vascular network in children. Our Chinese children, nevertheless, showed no association of a range of birth factors including pre-term birth and low birth weight with retinal vascular caliber, which was consistent with what was reported in Singapore children from SCORM in 2007.[45] It is possible that retinal vascular caliber may only reflect cumulative effects of systemic insults, and therefore retinal vascular caliber changes may not manifest at an early stage in life.

4.10 Associations between Retinal Microvasculature and Gestational Complications in Pregnant Women

Numerous cross-sectional and longitudinal studies have reported that retinal microvascular abnormalities are related to both present and future occurrence of systemic diseases such as hypertension and diabetes. Narrower retinal arteriolar caliber is associated with higher risk of incident hypertension after 3-10 years' follow-up in MESA, BDES, ARIC, BMES, the Rotterdam Study and the Funagata Study.[17, 155-158, 159]

The association between retinal microvasculature and incidence of metabolic diseases and related complications were also been widely studied. Narrower retinal arteriolar caliber, wider retinal venular caliber or smaller AVR were associated with future impaired fasting glucose and even diabetes in multiple population-based studies as BMES, AusDiab Study, the Rotterdam study and BDES.[160-163]

However, in our study, abnormal changes in retinal vascular caliber, retinal vascular tortuosity, retinal vascular branching angle and retinal vascular fractal dimension were not associated with onsets of gestational hypertension, pre-eclampsia or gestational diabetes mellitus (GDM). The reasons for the negative associations might lie in points listed below: Firstly, the cases for gestational hypertension, pre-eclampsia and chronic hypertension are as low as 20, 16 and 10, respectively. Such low sample size might bring insufficient study power to analyze the real association between retinal vascular parameters and gestational hypertensive disorders. Secondly, gestational diabetes mellitus (GDM) was diagnosed by the 26 weeks' oral glucose tolerance test (OGTT) and WHO classification guideline. Since the process of GDM is more transient than chronic diabetes, the temporary state of GDM might only cause retinal vascular functional changes rather than retinal vascular structural changes.

4.11 Strengths

Our study is one of the first to comprehensively investigate the relationships between major systematic factors and a series of retinal vascular parameters in pregnant women and very young children.

The high response rates for both original studies (76.3% in GUSTO and 72.3% in STARS), and high gradable percentage (97.5% in pregnant women and 93.0% in children) have reduced selection bias.

Secondly, quantitative measurements in our study have been conducted on pregnant women and children according to a series of standardized and validated protocols. For example, peripheral blood pressure (SBP and DBP) was measured by using the automatic Omron sphygmomanoter (Omron HEM 705 LP, Omron Healthcare Inc., US) and at least two readings along with an alternative third reading were required. The SECA (Vogel and Halke, Hamburg, Germany) weighing models were practiced in weight assessment for both populations. Subcutaneous skinfold thickness (triceps skinfold, biceps skinfold, subscapular skinfold and suprailiac skinfold) were measured by using the Holtain Tanner/Whitehouse skinfold caliper (Holtain Ltd, Crymych, Wales, UK). Ocular biometrics (axial length, corneal curvature and anterior chamber depth) were obtained monocularly by using the Zeiss IOLMaster (IOLMaster, Carl Zeiss, Germany) while autorefraction was performed by the Canon Autorefractor RF-F1 (Canon, Tokyo, Japan). Furthermore, retinal photos were taken by the Canon 45° digital retinal camera (Model CR6-NM45 and Model CR-1, Canon Inc., Japan). Retinal photographs were graded by trained staff by using widely recognized grading softwares—IVAN (the Interactive Vessel Analysis, University of Wisconsin, Madison, WI, US) and SIVA (Singapore I Vessel Assessment, Singapore Eye Research Institute, Singapore). Only grading results with high reproductively after intra-grader reliability test were further analyzed. WHO and CDC recognized definitions such as childhood hypertension, childhood obesity,

gestational hypertension, preeclampsia and maternal obesity were applied for our clinical classifications.

Thirdly, subjective assessments on antenatal mental health including symptoms of depression, symptoms of anxiety and sleep quality, a set of well validated caucasian-wise self-ministered questionnaires (the Edinburgh Postnatal Depression Scale [EPDS], the State-Trait Anxiety Inventory [STAI] and [26025425024025619119119190]the Pittsburg Sleeping Quality Index [PSQI]) were applied in our Asian population.

Last but not the least, detailed information on a range of potential confounders such as demographic information, family history, life style (smoking and alcohol drinking), medical history and birth parameters were collected.

4.12 Limitations

There are also several limitations in our study, which may potentially affect the true associations between risk factors and retinal microcirculation.

Firstly, selection bias in both children and pregnant women retinal studies may have affected our results, since only children who resided in HDB in south-western Singapore and pregnant women who attended KKH clinic were eligible.

Secondly, the cross-sectional nature of both children and pregnant women studies will not be able to establish the temporal sequence of a range of risk factors (e.g. blood pressure, body mass index, triceps skinfold, axial length, antenatal depressive symptoms and antenatal sleep quality) with retinal vascular parameters. For example,

the causality of elevated blood pressure and retinal arteriolar narrowing among pregnant women cannot be identified.

Thirdaly, the use of other body fatness indices instead of BMI is still debatable. TSF is a measurement for subcutaneous body fatness, which cannot substitute the internal body fatness. The real body fatness-retinal vascular caliber might not be fully revealed in our children retinal study. As for pregnant women, the lack of association of skinfold measurement and retinal microvasculature could be due to: 1. Gestational-related fatness is predominantly accumulated centrally; visceral fat might be a more preferential selection for central sites in pregnant women with high BMI than subcutaneous truncal fat[361, 362]. 2. Skinfold thickness in pregnant women measures both body fat deposit and interstitial body fluid, due to the hormone related edema condition. This may dilute the association towards the null value.

Fourthly, the self-administered psychological measures (EPDS, STAI and PSQI) have not been validated in our local pregnant women, and no structured diagnostic interviews were conducted. As the questionnaires were all filled in a single sitting, there could have been a response set bias.

Lastly, recall bias for pre-pregnancy weight may have influenced the association of pre-pregnancy BMI and weight gain with retinal vascular parameters. However recall of pregnancy weight has been used and validated in many other studies[363-365].

What's also worth mentioning is that this is the first attempt of retinal photography examination on very young children aged 4-5 years. Due to the poor cooperation (such as following instructions) of preschoolers, the quality of retinal photos was

affected. We were not able to grade retinal geometric parameters such as fractals, vessel tortuosity and branching angles as these assessments required gradable vessels in zone C area other than zone B area. Thus, only retinal vascular caliber in zone B area was available for grading in children retinal study. It would be of great interest to examine these associations if further research can be done to provide more parameters for assessing a complete retinal vascular geometry.

4.13 Significance

A body of evidence done by epidemiological studies has suggested that systematic diseases such as metabolic syndrome, hypertension and other cardiovascular risks may originate in early life[209, 210] and even in utero.[359, 360] Since we know the developments of metabolic diseases and vascular diseases are multi-factorially induced and regulated, the exposure of environmental factors and systematic factors in utero and in early life became essentially important to study pathophysiological mechanism for disease progression.

In Singapore multi-ethnic pregnant women, elevated blood pressure and greater maternal body mass index were associated with a range of adverse retinal vascular parameters changes, including retinal arteriolar narrowing, retinal venular widening, lower retinal arteriolar and venular tortuosity, lower retinal arteriolar and venular branching angle and lower retinal arteriolar fractal dimension. Antenatal depressive symptoms and poor sleep quality were only associated with retinal arteriolar widening. These findings biologically link up maternal blood pressure, maternal obesity and maternal negative mood with retinal microvasculature during mid-term pregnancy.

In Singapore Chinese children, elevated blood pressure was associated with retinal arteriolar narrowing. Greater body mass index and greater triceps skinfold thickness were associated with retinal venular widening. Longer axial length and greater corneal curvature were associated with retinal arteriolar and venular narrowing, which biologically link ocular axial dimension with retinal vasculature in very young children. These findings biologically link up childhood blood pressure, in-depth obesity and ocular axial dimension with retinal microvasculature in very young children.

Our study is the first study comprehensively investigating the relationships between systematic risk factors and microcirculation *in vivo* of early life and maternal/utero environments. In addition, our study helps to provide normative data of retinal vascular parameters in these two special populations, evidence that hypertension and obesity in childhood and in mid-term pregnancy are associated with vascular pathology, and further insights into pathophysiological mechanisms of metabolic and vascular diseases occurring in later life.

Since vascular pathology is speculated as the sequence of events leading to overt cardiovascular diseases which occurs typically later in adulthood or after delivery and the likelihood of reversibility of pathology following interventions, specialists like pediatricians and obstetricians should closely monitor retinal vasculature changes in pregnancy women and children, especially those with risks of elevated blood pressure, greater BMI, higher myopia, more depressive condition and poorer sleep quality in antenatal mental health throughout pregnancy and childhood. Retinal vascular assessment might be an effective means of approaching the public health campaigns

targeted on pregnancy women and children. Further longitudinal studies should work closely with physicians to better indicate counseling and begin intervention on appropriate retinal microvasculature changes in terms of its prediction on developments of metabolic disease and vascular diseases.

4.14 Future direction

This is the elevate from individual associations to a strategic point of view what implications this research has overall and where this work will lead to in further research and future clinical practice. Retinal vascular changes are not specific clinical conditions, but at the most, phenotypic variations which may or may not be clinically significant. After more than a decade of research in retinal imaging and retinal vascular morphological changes, some researchers suggested to revisit its significance and point out directions of the next steps of research in this field.

Based on the significant and interesting findings reported in our pregnant women cohort, it is unique to study the retinal micropathology in pregnant women and their descendants. Environmental influences, genetic influences and even epigenetic influences can be studied together with the phenotypic variation of microcirculation *in vivo*.

Furthermore, it is still useful to study retinal imaging and its relation to other systemic diseases such as auto-immune diseases and infectious diseases by linking endothelial dysfunction, oxidative stress and inflammation with retinal micropathology through etiological models and animal models. Furthermore, there are a lot of variables reported in the retinal imaging report, principle component

analysis (PCA) should be performed to determine which parameters are more related to which diseases. There are more and more examinations such as Oximap (visualizing oxygen transmissibility) and Dynamic Vessel Analysis (DVA), to test retinal circulation oxygen supply and dynamic vessel function. Future studies should try to combine the information provided by all these examinations, and to draw a more comprehensive conclusion on what role of retinal imaging is playing in systemic diseases and CVD.

Chapter 5

5. Epidemilogical Aspect of Our Study

As for the nature of cross-sectional design of our study, there would not be any causality deduced. However, in order to better evaluate our work from the epidemiological aspect, we further discuss three important components leading to systematic error, and resulting in affecting the validity of our findings.

5.1 Bias

Bias is a major issue for any kind of epidemiological designed study and defined as "any systematic error in the design, conduct or analysis of a study that results in a mistaken estimate of an exposure's effect on the risk of disease". As our retinal studies from children and pregnant women were recruited from two subsets from STARS/STARS Family study and GUSTO/GUSTO IVF study, separately, nonresponse of potential study subjects might cause our potential selection bias. In the STARS/STARS Family study, the original eligible subjects for retinal photography were up to 1776, however, there were only 608 turned up in our retinal sub-study with a final number of 586 participants with gradable retinal photos. First of all, the reason for such low response rate was due to the time of conducting our retinal study, which commenced in year 2009, which was also the last year for STARS and second year for STARS Family study at clinic. We tended to lose some of the potential cases and controls due to the low response rate. However, we calculated the effect size of our own study, the power turned up to be more than 0.85. Therefore, our children subset is representative for the orginal target population. As

for retinal study in pregnant women, the response rate of our sub-study was as high as 91.1%, effect size was calculated as high as 0.95. Again, our pregnant women subset is representative for the original target population. However, we didn't collect any data on the nonresponse participant, so that potential selection bias might exist. Since we don't know the distribution of cases and controls, exposed and non-exposed individuals in the nonresponse groups, external validity might be limited in our study.

As for information bias, it can occur when the means for obtaining information about the subjects in the study are inadequate, which results in some of the information gathered regarding exposures and/or disease outcome is incorrect. In order to minimize information bias, any type of clinical measurements were strictly following the standard protocol used in SCORM in Singapore and SCES in Australia. Also, questionnaire interviews were conducted by trained staff. Potential information errors were minimized by assigning double data entry.

5.2 Confounding

Confounding issue is one of the most important problems in observational epidemiological studies. Confounders can be adjusted in many ways, such as restriction, randomization, matching and stratification, etc. There are three criteria to define confounders, which are associated with exposure, associated with outcome and not on the causal pathway. If confounding is not adjusted for, the results will be spurious. For example, coffee consumption and pancreatic cancer are associated by clinical research. Yet smoking is a known risk factor for pancreatic cancer and also associated with coffee drinking too. Since smoking is not a result of coffee drinking,

the observed the association of coffee drinking and cancer of the pancreas may be a result of confounding by cigarette smoking. And when we want to find out the true association between coffee drinking and pancreatic cancer, we need to adjust for smoking.

In our study, we handled the confounding issue as such. Firstly, we performed bi-variate analysis for all potential independent variables and run it in a stepwise model to further determine relevant confounders for our linear regression model. However, in case there might be any residual confounder left, we would select other confounders which have been published in other studies earlier. Therefore, based on the statistical and clinical selection, we determined our final adjustment to count for confounding issue.

We mainly used multiple linear regression and multiple logistic regression, so collinearity is one of the major concern whether high inter-correlated variables might affect our findings. Therefore, we also ran simple collinearity test and multi-collinearity test in our analysis. The tolerance and variance inflation factor are both in accepted value range. As for blood pressure and body mass index, due to their biological relationship and widely used as confounders published in other clinical and epidemiological papers, we also tested the simple collinearity on these two variables. Even though the variance is more than 0.1, the magnitude of the association didn't change much by taking out one or the other from the original model. Also, residual plots were performed to check the validity of our multiple linear regression models. The pattern was normal horizontal plot. As we have controlled for possible biases and

addressed issues of confounding to avoid spurious results, our conclusions are more likely to be internally valid.

5.3 Interaction

Generally, there is always more than one factor involved in disease etiology. So how do multiple factors interact in causing a disease, we need to take interaction into account. Interaction also regarded as effect modification, which has been defined as "When the incidence rate of disease in the presence of two or more risk factors differs from the incidence rate expected to result from their individual effects" by MacMahon. Among our relationships found between blood pressure/body mass indices/psychosocial factors/ocular biometric factors and retinal vascular parameters, we tested all these associations in different stratas for example age groups, gender, ethnicity and smoking history. However, there was no effect modifier identified.

5.4 Study design

Our study design is observational and cross-sectional study, which could provide valuable information on etiology of the changes on retinal vascular parameters. However, the drawback of such study design is that, there is no causality deduced. As for case-control study, since the diseases have been selected, it is quick, less resource-intensive and possible to study rare disease. However, the temporal relationship is still difficult to establish, which is same as cross-sectional study. And recall bias and selection bias (for cases and controls) will affect the validity of the study. Unlike case-control study, cohort study can cover all the disadvantages occurring in cross-sectional study and cohort study. Temporal relationship can be established yet it

might be costly, time-consuming and lost in power with loss to follow-up. According to our study, we will be following up the pregnant women and their descendants annually, to collect medical information, physical measurements, change in life style and diet, together with retinal imaging, in order to study the role of retinal vascular morphology in systemic diseases and CVD development. Nested case-control study will be considered if our outcome is relatively rare diseases such as metabolic syndrome and type 2 diabetes among the 824 participants.

5.5 Type I and Type II Error and Significance

Type I error is when there is no true association yet we find in our study, while Type II error is when there is a true association yet we can't find in the study. First of all, our study looking for risk factors is a planned analysis rather than a fishing analysis, due to the fact that all these risk factors have been established to be associated with retinal vascular parameters in many epidemiological and clinical studies. Thus, bonferroni correction is not necessary in our analyses. We still set our p value as 0.05. When the p value reported in our study is less than 0.05, we are more likely to make Type I error, while p value reported is more than 0.05, we are more likely to make Type II error. However, for multiple comparisons like prevalence of hypertension/obesity/antennal depression across three ethnic groups among pregnant women, we used λ^2 test and multiple the p value with 3, to get a more conservative p value.

REFERENCES:

1. Sun C, Wang JJ, Mackey DA, Wong TY. Retinal vascular caliber: systemic, environmental, and genetic associations. *Surv Ophthalmol* 2009;54:74-95.
2. Wong TY. Is retinal photography useful in the measurement of stroke risk? *Lancet Neurol* 2004;3:179-183.
3. Wong TY, Mitchell P. The eye in hypertension. *Lancet* 2007;369:425-435.
4. Hubbard LD, Brothers RJ, King WN, et al. Methods for evaluation of retinal microvascular abnormalities associated with hypertension/sclerosis in the Atherosclerosis Risk in Communities Study. *Ophthalmology* 1999;106:2269-2280.
5. Cheung CY, Tay WT, Mitchell P, et al. Quantitative and qualitative retinal microvascular characteristics and blood pressure. *J Hypertens* 2011;29:1380-1391.
6. Gopinath B, Baur LA, Teber E, Liew G, Wong TY, Mitchell P. Effect of obesity on retinal vascular structure in pre-adolescent children. *Int J Pediatr Obes* 2011;6:e353-359.
7. Klein R, Klein BE, Moss SE, Wong TY, Sharrett AR. Retinal vascular caliber in persons with type 2 diabetes: the Wisconsin Epidemiological Study of Diabetic Retinopathy: XX. *Ophthalmology* 2006;113:1488-1498.
8. Sun C, Liew G, Wang JJ, et al. Retinal vascular caliber, blood pressure, and cardiovascular risk factors in an Asian population: the Singapore Malay Eye Study. *Invest Ophthalmol Vis Sci* 2008;49:1784-1790.
9. Wong TY, Islam FM, Klein R, et al. Retinal vascular caliber, cardiovascular risk factors, and inflammation: the multi-ethnic study of atherosclerosis (MESA). *Invest Ophthalmol Vis Sci* 2006;47:2341-2350.
10. Wong TY, Knudtson MD, Klein R, Klein BE, Meuer SM, Hubbard LD. Computer-assisted measurement of retinal vessel diameters in the Beaver Dam Eye Study: methodology, correlation between eyes, and effect of refractive errors. *Ophthalmology* 2004;111:1183-1190.
11. Cheung CY, Zheng Y, Hsu W, et al. Retinal vascular tortuosity, blood pressure, and cardiovascular risk factors. *Ophthalmology* 2011;118:812-818.
12. Cheung N, Liew G, Lindley RI, et al. Retinal fractals and acute lacunar stroke. *Ann Neurol* 2010;68:107-111.
13. Cooper LS, Wong TY, Klein R, et al. Retinal microvascular abnormalities and MRI-defined subclinical cerebral infarction: the Atherosclerosis Risk in Communities Study. *Stroke* 2006;37:82-86.
14. Sabanayagam C, Shankar A, Koh D, et al. Retinal microvascular caliber and chronic kidney disease in an Asian population. *Am J Epidemiol* 2009;169:625-632.
15. Sasongko MB, Wong TY, Donaghue KC, et al. Retinal arteriolar tortuosity is associated with retinopathy and early kidney dysfunction in type 1 diabetes. *Am J Ophthalmol* 2012;153:176-183 e171.
16. Tikellis G, Arnett DK, Skelton TN, et al. Retinal arteriolar narrowing and left ventricular hypertrophy in African Americans. the Atherosclerosis Risk in Communities (ARIC) study. *Am J Hypertens* 2008;21:352-359.
17. Ikram MK, Witteman JC, Vingerling JR, Breteler MM, Hofman A, de Jong PT. Retinal vessel diameters and risk of hypertension: the Rotterdam Study. *Hypertension* 2006;47:189-194.
18. Parr JC, Spears GF. Mathematic relationships between the width of a retinal artery and the widths of its branches. *Am J Ophthalmol* 1974;77:478-483.

19. Parr JC, Spears GF. General caliber of the retinal arteries expressed as the equivalent width of the central retinal artery. *Am J Ophthalmol* 1974;77:472-477.
20. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BE. Revised formulas for summarizing retinal vessel diameters. *Curr Eye Res* 2003;27:143-149.
21. Mahal S, Strain WD, Martinez-Perez ME, Thom SA, Chaturvedi N, Hughes AD. Comparison of the retinal microvasculature in European and African-Caribbean people with diabetes. *Clin Sci (Lond)* 2009;117:229-236.
22. Owen CG, Rudnicka AR, Nightingale CM, et al. Retinal arteriolar tortuosity and cardiovascular risk factors in a multi-ethnic population study of 10-year-old children; the Child Heart and Health Study in England (CHASE). *Arterioscler Thromb Vasc Biol* 2011;31:1933-1938.
23. Gopinath B, Baur LA, Pfund N, Burlutsky G, Mitchell P. Differences in association between birth parameters and blood pressure in children from preschool to high school. *J Hum Hypertens* 2012.
24. Gopinath B, Flood VM, Rochtchina E, Baur LA, Smith W, Mitchell P. Influence of High Glycemic Index and Glycemic Load Diets on Blood Pressure During Adolescence. *Hypertension* 2012.
25. Gopinath B, Flood VM, Wang JJ, et al. Carbohydrate nutrition is associated with changes in the retinal vascular structure and branching pattern in children. *Am J Clin Nutr* 2012;95:1215-1222.
26. Gopinath B, Schneider J, Hickson L, et al. Hearing handicap, rather than measured hearing impairment, predicts poorer quality of life over 10 years in older adults. *Maturitas* 2012;72:146-151.
27. Hanssen H, Siegrist M, Neidig M, et al. Retinal vessel diameter, obesity and metabolic risk factors in school children (JuvenTUM 3). *Atherosclerosis* 2012;221:242-248.
28. Liew G, Sharrett AR, Wang JJ, et al. Relative importance of systemic determinants of retinal arteriolar and venular caliber: the atherosclerosis risk in communities study. *Arch Ophthalmol* 2008;126:1404-1410.
29. Wong TY, Klein R, Klein BE, Meuer SM, Hubbard LD. Retinal vessel diameters and their associations with age and blood pressure. *Invest Ophthalmol Vis Sci* 2003;44:4644-4650.
30. Wong TY, Klein R, Sharrett AR, et al. Retinal arteriolar narrowing and risk of diabetes mellitus in middle-aged persons. *JAMA* 2002;287:2528-2533.
31. Li LJ, Cheung CY, Liu Y, et al. Influence of blood pressure on retinal vascular caliber in young children. *Ophthalmology* 2011;118:1459-1465.
32. Murray CD. The Physiological Principle of Minimum Work: II. Oxygen Exchange in Capillaries. *Proc Natl Acad Sci U S A* 1926;12:299-304.
33. Changizi MA, Cherniak C. Modeling the large-scale geometry of human coronary arteries. *Can J Physiol Pharmacol* 2000;78:603-611.
34. Rossitti S, Lofgren J. Optimality principles and flow orderliness at the branching points of cerebral arteries. *Stroke* 1993;24:1029-1032.
35. Liew G, Wang JJ, Cheung N, et al. The retinal vasculature as a fractal: methodology, reliability, and relationship to blood pressure. *Ophthalmology* 2008;115:1951-1956.
36. Patton N, Aslam TM, MacGillivray T, et al. Retinal image analysis: concepts, applications and potential. *Prog Retin Eye Res* 2006;25:99-127.
37. Gepstein R, Rosman Y, Rechtman E, et al. Association of retinal microvascular caliber with blood pressure levels. *Blood Press* 2012;21:191-196.

38. Kawasaki R, Wang JJ, Rochtchina E, et al. Cardiovascular risk factors and retinal microvascular signs in an adult Japanese population: the Funagata Study. *Ophthalmology* 2006;113:1378-1384.
39. Leung H, Wang JJ, Rochtchina E, et al. Relationships between age, blood pressure, and retinal vessel diameters in an older population. *Invest Ophthalmol Vis Sci* 2003;44:2900-2904.
40. Doubal FN, MacGillivray TJ, Patton N, Dhillon B, Dennis MS, Wardlaw JM. Fractal analysis of retinal vessels suggests that a distinct vasculopathy causes lacunar stroke. *Neurology* 2010;74:1102-1107.
41. Liew G, Mitchell P, Rochtchina E, et al. Fractal analysis of retinal microvasculature and coronary heart disease mortality. *Eur Heart J* 2011;32:422-429.
42. Sasongko MB, Wong TY, Nguyen TT, Cheung CY, Shaw JE, Wang JJ. Retinal vascular tortuosity in persons with diabetes and diabetic retinopathy. *Diabetologia* 2011;54:2409-2416.
43. Wong TY, Knudtson MD, Klein BE, Klein R, Hubbard LD. Estrogen replacement therapy and retinal vascular caliber. *Ophthalmology* 2005;112:553-558.
44. Rochtchina E, Wang JJ, Taylor B, Wong TY, Mitchell P. Ethnic variability in retinal vessel caliber: a potential source of measurement error from ocular pigmentation?--the Sydney Childhood Eye Study. *Invest Ophthalmol Vis Sci* 2008;49:1362-1366.
45. Cheung N, Islam FM, Saw SM, et al. Distribution and associations of retinal vascular caliber with ethnicity, gender, and birth parameters in young children. *Invest Ophthalmol Vis Sci* 2007;48:1018-1024.
46. Jensen RA, Shea S, Ranjit N, et al. Psychosocial risk factors and retinal microvascular signs: the multi-ethnic study of atherosclerosis. *Am J Epidemiol* 2010;171:522-531.
47. Kifley A, Liew G, Wang JJ, et al. Long-term effects of smoking on retinal microvascular caliber. *Am J Epidemiol* 2007;166:1288-1297.
48. Klein R, Klein BE, Moss SE, Wong TY. Retinal vessel caliber and microvascular and macrovascular disease in type 2 diabetes: XXI: the Wisconsin Epidemiologic Study of Diabetic Retinopathy. *Ophthalmology* 2007;114:1884-1892.
49. Andersen LB, Riddoch C, Kriemler S, Hills AP. Physical activity and cardiovascular risk factors in children. *Br J Sports Med* 2011;45:871-876.
50. Church T. Exercise in obesity, metabolic syndrome, and diabetes. *Prog Cardiovasc Dis* 2011;53:412-418.
51. Guinhouya BC, Samouda H, Zitouni D, Vilhelm C, Hubert H. Evidence of the influence of physical activity on the metabolic syndrome and/or on insulin resistance in pediatric populations: a systematic review. *Int J Pediatr Obes* 2011;6:361-388.
52. Scott D, Happell B. The high prevalence of poor physical health and unhealthy lifestyle behaviours in individuals with severe mental illness. *Issues Ment Health Nurs* 2011;32:589-597.
53. Anuradha S, Healy GN, Dunstan DW, et al. Physical activity, television viewing time, and retinal microvascular caliber: the multi-ethnic study of atherosclerosis. *Am J Epidemiol* 2011;173:518-525.
54. Anuradha S, Healy GN, Dunstan DW, et al. Associations of physical activity and television viewing time with retinal vascular caliber in a multiethnic Asian population. *Invest Ophthalmol Vis Sci* 2011;52:6522-6528.
55. de Jong FJ, Vernooij MW, Ikram MK, et al. Arteriolar oxygen saturation, cerebral blood flow, and retinal vessel diameters. The Rotterdam Study. *Ophthalmology* 2008;115:887-892.

56. Gopinath B, Baur LA, Wang JJ, et al. Influence of physical activity and screen time on the retinal microvasculature in young children. *Arterioscler Thromb Vasc Biol* 2011;31:1233-1239.
57. Hanssen H, Nickel T, Drexel V, et al. Exercise-induced alterations of retinal vessel diameters and cardiovascular risk reduction in obesity. *Atherosclerosis* 2011;216:433-439.
58. Tikellis G, Anuradha S, Klein R, Wong TY. Association between physical activity and retinal microvascular signs: the Atherosclerosis Risk in Communities (ARIC) Study. *Microcirculation* 2010;17:381-393.
59. Jeganathan VS, Sabanayagam C, Tai ES, et al. Effect of blood pressure on the retinal vasculature in a multi-ethnic Asian population. *Hypertens Res* 2009;32:975-982.
60. Wong TY, McIntosh R. Hypertensive retinopathy signs as risk indicators of cardiovascular morbidity and mortality. *Br Med Bull* 2005;73-74:57-70.
61. Gopinath B, Baur LA, Wang JJ, et al. Blood pressure is associated with retinal vessel signs in preadolescent children. *J Hypertens* 2010;28:1406-1412.
62. Mitchell P, Cheung N, de Haseth K, et al. Blood pressure and retinal arteriolar narrowing in children. *Hypertension* 2007;49:1156-1162.
63. Liew G, Wong TY, Mitchell P, Wang JJ. Are narrower or wider retinal venules associated with incident hypertension? *Hypertension* 2006;48:e10; author reply e11.
64. Gopinath B, Baur LA, Hardy LL, et al. Parental history of hypertension is associated with narrower retinal arteriolar caliber in young girls. *Hypertension* 2011;58:425-430.
65. Ikram MK, de Jong FJ, Vingerling JR, et al. Are retinal arteriolar or venular diameters associated with markers for cardiovascular disorders? The Rotterdam Study. *Invest Ophthalmol Vis Sci* 2004;45:2129-2134.
66. Kaushik S, Kifley A, Mitchell P, Wang JJ. Age, blood pressure, and retinal vessel diameter: separate effects and interaction of blood pressure and age. *Invest Ophthalmol Vis Sci* 2007;48:557-561.
67. Taarnhoj NC, Munch IC, Sander B, et al. Straight versus tortuous retinal arteries in relation to blood pressure and genetics. *Br J Ophthalmol* 2008;92:1055-1060.
68. Sasongko MB, Wong TY, Donaghue KC, et al. Retinal arteriolar tortuosity is associated with retinopathy and early kidney dysfunction in type 1 diabetes. *Am J Ophthalmol* 153:176-183 e171.
69. Owen CG, Rudnicka AR, Nightingale CM, et al. Retinal arteriolar tortuosity and cardiovascular risk factors in a multi-ethnic population study of 10-year-old children; the Child Heart and Health Study in England (CHASE). *Arterioscler Thromb Vasc Biol* 31:1933-1938.
70. Owen CG, Newsom RS, Rudnicka AR, Barman SA, Woodward EG, Ellis TJ. Diabetes and the tortuosity of vessels of the bulbar conjunctiva. *Ophthalmology* 2008;115:e27-32.
71. Kurniawan ED, Cheung N, Cheung CY, Tay WT, Saw SM, Wong TY. Elevated blood pressure is associated with rarefaction of the retinal vasculature in children. *Invest Ophthalmol Vis Sci* 53:470-474.
72. Cheung CY, Zheng Y, Hsu W, et al. Retinal vascular tortuosity, blood pressure, and cardiovascular risk factors. *Ophthalmology* 118:812-818.
73. Gopinath B, Baur LA, Wang JJ, et al. Blood pressure is associated with retinal vessel signs in preadolescent children. *J Hypertens* 2010.
74. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser* 2000;894:i-xii, 1-253.
75. Cheung N, Wong TY. Obesity and eye diseases. *Surv Ophthalmol* 2007;52:180-195.

76. Taylor B, Rochtchina E, Wang JJ, et al. Body mass index and its effects on retinal vessel diameter in 6-year-old children. *Int J Obes (Lond)* 2007;31:1527-1533.
77. Kawasaki R, Tielsch JM, Wang JJ, et al. The metabolic syndrome and retinal microvascular signs in a Japanese population: the Funagata study. *Br J Ophthalmol* 2008;92:161-166.
78. Klein BE, Moss SE, Klein R, Surawicz TS. The Wisconsin Epidemiologic Study of Diabetic Retinopathy. XIII. Relationship of serum cholesterol to retinopathy and hard exudate. *Ophthalmology* 1991;98:1261-1265.
79. Ross R. Atherosclerosis is an inflammatory disease. *Am Heart J* 1999;138:S419-420.
80. Van Hecke MV, Dekker JM, Nijpels G, et al. Homocysteine, S-adenosylmethionine and S-adenosylhomocysteine are associated with retinal microvascular abnormalities: the Hoorn Study. *Clin Sci (Lond)* 2008;114:479-487.
81. Yim-Lui Cheung C, Wong TY, Lamoureux EL, et al. C-reactive protein and retinal microvascular caliber in a multiethnic asian population. *Am J Epidemiol* 171:206-213.
82. Gopinath B, Wang JJ, Flood VM, Burlutsky G, Wong TY, Mitchell P. The associations between blood levels of homocysteine, folate, vitamin B12, and retinal vascular caliber. *Am J Ophthalmol* 2009;148:902-909.
83. Klein R, Klein BE, Knudtson MD, Wong TY, Tsai MY. Are inflammatory factors related to retinal vessel caliber? The Beaver Dam Eye Study. *Arch Ophthalmol* 2006;124:87-94.
84. Stettler C, Witt N, Tapp RJ, et al. Serum amyloid A, C-reactive protein, and retinal microvascular changes in hypertensive diabetic and nondiabetic individuals: an Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) substudy. *Diabetes Care* 2009;32:1098-1100.
85. Zheng Y, Cheung N, Aung T, Mitchell P, He M, Wong TY. Relationship of retinal vascular caliber with retinal nerve fiber layer thickness: the singapore malay eye study. *Invest Ophthalmol Vis Sci* 2009;50:4091-4096.
86. Chang M, Yoo C, Kim SW, Kim YY. Retinal vessel diameter, retinal nerve fiber layer thickness, and intraocular pressure in korean patients with normal-tension glaucoma. *Am J Ophthalmol* 151:100-105 e101.
87. Cheung N, Huynh S, Wang JJ, et al. Relationships of retinal vessel diameters with optic disc, macular and retinal nerve fiber layer parameters in 6-year-old children. *Invest Ophthalmol Vis Sci* 2008;49:2403-2408.
88. Cheung N, Tikellis G, Saw SM, et al. Relationship of axial length and retinal vascular caliber in children. *Am J Ophthalmol* 2007;144:658-662.
89. Cheung N, Tong L, Tikellis G, et al. Relationship of retinal vascular caliber with optic disc diameter in children. *Invest Ophthalmol Vis Sci* 2007;48:4945-4948.
90. Koh V, Cheung CY, Zheng Y, Wong TY, Wong W, Aung T. Relationship of retinal vascular tortuosity with the neuroretinal rim: the singapore malay eye study. *Invest Ophthalmol Vis Sci* 51:3736-3741.
91. Lim L, Cheung N, Gazzard G, Chan YH, Wong TY, Saw SM. Corneal biomechanical properties and retinal vascular caliber in children. *Invest Ophthalmol Vis Sci* 2009;50:121-125.
92. Lim LS, Cheung CY, Lin X, Mitchell P, Wong TY, Mei-Saw S. Influence of refractive error and axial length on retinal vessel geometric characteristics. *Invest Ophthalmol Vis Sci* 52:669-678.
93. Lim LS, Saw SM, Cheung N, Mitchell P, Wong TY. Relationship of retinal vascular caliber with optic disc and macular structure. *Am J Ophthalmol* 2009;148:368-375.

94. Samarawickrama C, Huynh SC, Wang JJ, et al. Relationship between retinal structures and retinal vessel caliber in normal adolescents. *Invest Ophthalmol Vis Sci* 2009;50:5619-5624.
95. Wong TY, Wang JJ, Rochtchina E, Klein R, Mitchell P. Does refractive error influence the association of blood pressure and retinal vessel diameters? The Blue Mountains Eye Study. *Am J Ophthalmol* 2004;137:1050-1055.
96. Avetisov ES, Savitskaya NF. Some features of ocular microcirculation in myopia. *Ann Ophthalmol* 1977;9:1261-1264.
97. Benavente-Perez A, Hosking SL, Logan NS, Broadway DC. Ocular blood flow measurements in healthy human myopic eyes. *Graefes Arch Clin Exp Ophthalmol* 248:1587-1594.
98. Perkins ES. The ocular pulse. *Curr Eye Res* 1981;1:19-23.
99. Tsai AS, Wong TY, Lavanya R, et al. Differential association of retinal arteriolar and venular caliber with diabetes and retinopathy. *Diabetes Res Clin Pract* 94:291-298.
100. Jeganathan VS, Sabanayagam C, Tai ES, et al. Retinal vascular caliber and diabetes in a multiethnic Asian population. *Microcirculation* 2009;16:534-543.
101. Nguyen TT, Wang JJ, Sharrett AR, et al. Relationship of retinal vascular caliber with diabetes and retinopathy: the Multi-Ethnic Study of Atherosclerosis (MESA). *Diabetes Care* 2008;31:544-549.
102. Kifley A, Wang JJ, Cugati S, Wong TY, Mitchell P. Retinal vascular caliber, diabetes, and retinopathy. *Am J Ophthalmol* 2007;143:1024-1026.
103. Bronson-Castain KW, Bearse MA, Jr., Neuville J, et al. Adolescents with Type 2 diabetes: early indications of focal retinal neuropathy, retinal thinning, and venular dilation. *Retina* 2009;29:618-626.
104. Yau JW, Kawasaki R, Islam FM, et al. Retinal fractal dimension is increased in persons with diabetes but not impaired glucose metabolism: the Australian Diabetes, Obesity and Lifestyle (AusDiab) study. *Diabetologia* 53:2042-2045.
105. Yuan Y, Ikram MK, Vingerling JR, et al. Retinal Vascular Caliber and Metabolic Syndrome in a Chinese Population. *Intern Med J*.
106. Wong TY, Duncan BB, Golden SH, et al. Associations between the metabolic syndrome and retinal microvascular signs: the Atherosclerosis Risk In Communities study. *Invest Ophthalmol Vis Sci* 2004;45:2949-2954.
107. Saito K, Nagao Y, Yamashita H, Kawasaki R. Screening for retinal vessel caliber and its association with metabolic syndrome in Japanese adults. *Metab Syndr Relat Disord* 9:427-432.
108. Rockwood K, Wentzel C, Hachinski V, Hogan DB, MacKnight C, McDowell I. Prevalence and outcomes of vascular cognitive impairment. Vascular Cognitive Impairment Investigators of the Canadian Study of Health and Aging. *Neurology* 2000;54:447-451.
109. Hachinski V, Munoz DG. Cerebrovascular pathology in Alzheimer's disease: cause, effect or epiphenomenon? *Ann N Y Acad Sci* 1997;826:1-6.
110. Berisha F, Feke GT, Trempe CL, McMeel JW, Schepens CL. Retinal abnormalities in early Alzheimer's disease. *Invest Ophthalmol Vis Sci* 2007;48:2285-2289.
111. Baker ML, Marino Larsen EK, Kuller LH, et al. Retinal microvascular signs, cognitive function, and dementia in older persons: the Cardiovascular Health Study. *Stroke* 2007;38:2041-2047.
112. Sabanayagam C, Tai ES, Lee J, Lim SC, Wong TY. Retinal vessel caliber and peripheral neuropathy in diabetic participants. *Microcirculation* 17:297-302.

113. Liew G, Mitchell P, Wong TY, Wang JJ. Retinal vascular caliber and migraine: the Blue Mountains Eye Study. *Headache* 2006;46:997-1004.
114. Cheung N, Rogers S, Mosley TH, Klein R, Couper D, Wong TY. Vital exhaustion and retinal microvascular changes in cardiovascular disease: atherosclerosis risk in communities study. *Psychosom Med* 2009;71:308-312.
115. Kaushik S, Wang JJ, Wong TY, et al. Glycemic index, retinal vascular caliber, and stroke mortality. *Stroke* 2009;40:206-212.
116. Kaushik S, Wang JJ, Flood V, Liew G, Smith W, Mitchell P. Frequency of fish consumption, retinal microvascular signs and vascular mortality. *Microcirculation* 2008;15:27-36.
117. Kan H, Stevens J, Heiss G, Klein R, Rose KM, London SJ. Dietary fiber intake and retinal vascular caliber in the Atherosclerosis Risk in Communities Study. *Am J Clin Nutr* 2007;86:1626-1632.
118. Wong TY, Knudtson MD, Klein BE, Klein R, Hubbard LD. Medication use and retinal vessel diameters. *Am J Ophthalmol* 2005;139:373-375.
119. Wickremasinghe SS, Rogers SL, Gillies MC, Zhu M, Wong TY. Retinal vascular caliber changes after intravitreal triamcinolone treatment for diabetic macular edema. *Invest Ophthalmol Vis Sci* 2008;49:4707-4711.
120. Metelitsina TI, Grunwald JE, DuPont JC, Ying GS, Liu C. Effect of viagra on retinal vein diameter in AMD patients. *Exp Eye Res* 2006;83:128-132.
121. Liew G, Mitchell P, Leeder SR, Smith W, Wong TY, Wang JJ. Regular aspirin use and retinal microvascular signs: the Blue Mountains Eye Study. *J Hypertens* 2006;24:1329-1335.
122. Leung H, Wang JJ, Rochtchina E, Wong TY, Klein R, Mitchell P. Does hormone replacement therapy influence retinal microvascular caliber? *Microvasc Res* 2004;67:48-54.
123. Barakat MR, Metelitsina TI, DuPont JC, Grunwald JE. Effect of niacin on retinal vascular diameter in patients with age-related macular degeneration. *Curr Eye Res* 2006;31:629-634.
124. Leung IY, Lai S, Ren S, et al. Early retinal vascular abnormalities in African-American cocaine users. *Am J Ophthalmol* 2008;146:612-619.
125. Wang JJ, Mitchell P, Leung H, Rochtchina E, Wong TY, Klein R. Hypertensive retinal vessel wall signs in a general older population: the Blue Mountains Eye Study. *Hypertension* 2003;42:534-541.
126. Zhou P, Wang M, Cao H. Research on features of retinal images associated with hypertension and diabetes. *Conf Proc IEEE Eng Med Biol Soc* 2005;6:6415-6417.
127. Hughes AD, Martinez-Perez E, Jabbar AS, et al. Quantification of topological changes in retinal vascular architecture in essential and malignant hypertension. *J Hypertens* 2006;24:889-894.
128. Nguyen TT, Islam FM, Farouque HM, et al. Retinal vascular caliber and brachial flow-mediated dilation: the Multi-Ethnic Study of Atherosclerosis. *Stroke* 41:1343-1348.
129. Pressler A, Esefeld K, Scherr J, et al. Structural alterations of retinal arterioles in adults late after repair of aortic isthmic coarctation. *Am J Cardiol* 105:740-744.
130. Cheung N, Islam FM, Jacobs DR, Jr., et al. Arterial compliance and retinal vascular caliber in cerebrovascular disease. *Ann Neurol* 2007;62:618-624.
131. Cheung N, Sharrett AR, Klein R, et al. Aortic distensibility and retinal arteriolar narrowing: the multi-ethnic study of atherosclerosis. *Hypertension* 2007;50:617-622.
132. van Hecke MV, Dekker JM, Nijpels G, et al. Are retinal microvascular abnormalities associated with large artery endothelial dysfunction and intima-media thickness? The Hoorn Study. *Clin Sci (Lond)* 2006;110:597-604.

133. De Silva DA, Liew G, Wong MC, et al. Retinal vascular caliber and extracranial carotid disease in patients with acute ischemic stroke: the Multi-Centre Retinal Stroke (MCRS) study. *Stroke* 2009;40:3695-3699.
134. Cheung N, Bluemke DA, Klein R, et al. Retinal arteriolar narrowing and left ventricular remodeling: the multi-ethnic study of atherosclerosis. *J Am Coll Cardiol* 2007;50:48-55.
135. Wang L, Wong TY, Sharrett AR, Klein R, Folsom AR, Jerosch-Herold M. Relationship between retinal arteriolar narrowing and myocardial perfusion: multi-ethnic study of atherosclerosis. *Hypertension* 2008;51:119-126.
136. Tsui I, Shamsa K, Perloff JK, Lee E, Wirthlin RS, Schwartz SD. Retinal vascular patterns in adults with cyanotic congenital heart disease. *Semin Ophthalmol* 2009;24:262-265.
137. Doubal FN, MacGillivray TJ, Patton N, Dhillon B, Dennis MS, Wardlaw JM. Fractal analysis of retinal vessels suggests that a distinct vasculopathy causes lacunar stroke. *Neurology* 74:1102-1107.
138. Cheung N, Liew G, Lindley RI, et al. Retinal fractals and acute lacunar stroke. *Ann Neurol* 68:107-111.
139. Baker ML, Wang JJ, Liew G, et al. Differential associations of cortical and subcortical cerebral atrophy with retinal vascular signs in patients with acute stroke. *Stroke* 41:2143-2150.
140. Roine S, Harju M, Kivela TT, et al. Ophthalmologic findings in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: a cross-sectional study. *Ophthalmology* 2006;113:1411-1417.
141. Cavallari M, Falco T, Frontali M, Romano S, Bagnato F, Orzi F. Fractal analysis reveals reduced complexity of retinal vessels in CADASIL. *PLoS One* 6:e19150.
142. Youm DJ, Ha MM, Chang Y, Song SJ. Retinal vessel caliber and risk factors for branch retinal vein occlusion. *Curr Eye Res* 37:334-338.
143. Tikellis G, Gillies MC, Guymer RH, McAllister IL, Shaw JE, Wong TY. Retinal vascular caliber and macular telangiectasia type 2. *Ophthalmology* 2009;116:319-323.
144. Gluckman PD, Hanson MA. Living with the past: evolution, development, and patterns of disease. *Science* 2004;305:1733-1736.
145. Bateson P, Barker D, Clutton-Brock T, et al. Developmental plasticity and human health. *Nature* 2004;430:419-421.
146. Barker DJ, Bagby SP. Developmental antecedents of cardiovascular disease: a historical perspective. *J Am Soc Nephrol* 2005;16:2537-2544.
147. Taylor SJ, Whincup PH, Cook DG, Papacosta O, Walker M. Size at birth and blood pressure: cross sectional study in 8-11 year old children. *BMJ* 1997;314:475-480.
148. Davies AA, Smith GD, May MT, Ben-Shlomo Y. Association between birth weight and blood pressure is robust, amplifies with age, and may be underestimated. *Hypertension* 2006;48:431-436.
149. Barker DJ, Osmond C, Simmonds SJ, Wield GA. The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. *BMJ* 1993;306:422-426.
150. Sun C, Ponsonby AL, Wong TY, et al. Effect of birth parameters on retinal vascular caliber: the Twins Eye Study in Tasmania. *Hypertension* 2009;53:487-493.
151. Mitchell P, Liew G, Rochtchina E, et al. Evidence of arteriolar narrowing in low-birth-weight children. *Circulation* 2008;118:518-524.
152. Liew G, Wang JJ, Duncan BB, et al. Low birthweight is associated with narrower arterioles in adults. *Hypertension* 2008;51:933-938.

153. Gopinath B, Baur LA, Wang JJ, et al. Smaller birth size is associated with narrower retinal arterioles in early adolescence. *Microcirculation* 17:660-668.
154. Cheung N, Wong TY, Liew G, Saw SM. Low birth weight and retinal vascular caliber in young children. *Pediatrics* 2008;121:862-863; author reply 863.
155. Kawasaki R, Cheung N, Wang JJ, et al. Retinal vessel diameters and risk of hypertension: the Multiethnic Study of Atherosclerosis. *J Hypertens* 2009;27:2386-2393.
156. Wong TY, Shankar A, Klein R, Klein BE, Hubbard LD. Prospective cohort study of retinal vessel diameters and risk of hypertension. *BMJ* 2004;329:79.
157. Wong TY, Klein R, Sharrett AR, et al. Retinal arteriolar diameter and risk for hypertension. *Ann Intern Med* 2004;140:248-255.
158. Tanabe Y, Kawasaki R, Wang JJ, et al. Retinal arteriolar narrowing predicts 5-year risk of hypertension in Japanese people: the Funagata study. *Microcirculation* 17:94-102.
159. Smith W, Wang JJ, Wong TY, et al. Retinal arteriolar narrowing is associated with 5-year incident severe hypertension: the Blue Mountains Eye Study. *Hypertension* 2004;44:442-447.
160. Wong TY, Mohamed Q, Klein R, Couper DJ. Do retinopathy signs in non-diabetic individuals predict the subsequent risk of diabetes? *Br J Ophthalmol* 2006;90:301-303.
161. Nguyen TT, Wang JJ, Islam FM, et al. Retinal arteriolar narrowing predicts incidence of diabetes: the Australian Diabetes, Obesity and Lifestyle (AusDiab) Study. *Diabetes* 2008;57:536-539.
162. Kifley A, Wang JJ, Cugati S, Wong TY, Mitchell P. Retinal vascular caliber and the long-term risk of diabetes and impaired fasting glucose: the Blue Mountains Eye Study. *Microcirculation* 2008;15:373-377.
163. Ikram MK, Janssen JA, Roos AM, et al. Retinal vessel diameters and risk of impaired fasting glucose or diabetes: the Rotterdam study. *Diabetes* 2006;55:506-510.
164. Roy MS, Klein R, Janal MN. Retinal venular diameter as an early indicator of progression to proliferative diabetic retinopathy with and without high-risk characteristics in African Americans with type 1 diabetes mellitus. *Arch Ophthalmol* 129:8-15.
165. Rogers SL, Tikellis G, Cheung N, et al. Retinal arteriolar caliber predicts incident retinopathy: the Australian Diabetes, Obesity and Lifestyle (AusDiab) study. *Diabetes Care* 2008;31:761-763.
166. Klein R, Klein BE, Moss SE, et al. The relation of retinal vessel caliber to the incidence and progression of diabetic retinopathy: XIX: the Wisconsin Epidemiologic Study of Diabetic Retinopathy. *Arch Ophthalmol* 2004;122:76-83.
167. Alibrahim E, Donaghue KC, Rogers S, et al. Retinal vascular caliber and risk of retinopathy in young patients with type 1 diabetes. *Ophthalmology* 2006;113:1499-1503.
168. Cheung N, Rogers SL, Donaghue KC, Jenkins AJ, Tikellis G, Wong TY. Retinal arteriolar dilation predicts retinopathy in adolescents with type 1 diabetes. *Diabetes Care* 2008;31:1842-1846.
169. Benitez-Aguirre P, Craig ME, Sasongko MB, et al. Retinal vascular geometry predicts incident retinopathy in young people with type 1 diabetes: a prospective cohort study from adolescence. *Diabetes Care* 34:1622-1627.
170. Yau JW, Xie J, Kawasaki R, et al. Retinal arteriolar narrowing and subsequent development of CKD Stage 3: the Multi-Ethnic Study of Atherosclerosis (MESA). *Am J Kidney Dis* 58:39-46.
171. Wong TY, Shankar A, Klein R, Klein BE. Retinal vessel diameters and the incidence of gross proteinuria and renal insufficiency in people with type 1 diabetes. *Diabetes* 2004;53:179-184.

172. Benitez-Aguirre PZ, Sasongko MB, Craig ME, et al. Retinal vascular geometry predicts incident renal dysfunction in young people with type 1 diabetes. *Diabetes Care* 35:599-604.
173. Wong TY, Klein R, Sharrett AR, et al. Retinal arteriolar narrowing and risk of coronary heart disease in men and women. The Atherosclerosis Risk in Communities Study. *JAMA* 2002;287:1153-1159.
174. Wong TY, Kamineni A, Klein R, et al. Quantitative retinal venular caliber and risk of cardiovascular disease in older persons: the cardiovascular health study. *Arch Intern Med* 2006;166:2388-2394.
175. Miller RG, Prince CT, Klein R, Orchard TJ. Retinal vessel diameter and the incidence of coronary artery disease in type 1 diabetes. *Am J Ophthalmol* 2009;147:653-660.
176. McGeechan K, Liew G, Macaskill P, et al. Risk prediction of coronary heart disease based on retinal vascular caliber (from the Atherosclerosis Risk In Communities [ARIC] Study). *Am J Cardiol* 2008;102:58-63.
177. Wang JJ, Liew G, Klein R, et al. Retinal vessel diameter and cardiovascular mortality: pooled data analysis from two older populations. *Eur Heart J* 2007;28:1984-1992.
178. Wong TY, Klein R, Nieto FJ, et al. Retinal microvascular abnormalities and 10-year cardiovascular mortality: a population-based case-control study. *Ophthalmology* 2003;110:933-940.
179. Liew G, Mitchell P, Rochtchina E, et al. Fractal analysis of retinal microvasculature and coronary heart disease mortality. *Eur Heart J* 32:422-429.
180. Kwa VI, van der Sande JJ, Stam J, Tijmes N, Vrooland JL. Retinal arterial changes correlate with cerebral small-vessel disease. *Neurology* 2002;59:1536-1540.
181. Ikram MK, De Jong FJ, Van Dijk EJ, et al. Retinal vessel diameters and cerebral small vessel disease: the Rotterdam Scan Study. *Brain* 2006;129:182-188.
182. de Jong FJ, Schrijvers EM, Ikram MK, et al. Retinal vascular caliber and risk of dementia: the Rotterdam study. *Neurology* 76:816-821.
183. Gangaputra S, Kalyani PS, Fawzi AA, et al. Retinal vessel caliber among people with acquired immunodeficiency syndrome: relationships with disease-associated factors and mortality. *Am J Ophthalmol* 153:434-444 e431.
184. Ikram MK, de Voogd S, Wolfs RC, et al. Retinal vessel diameters and incident open-angle glaucoma and optic disc changes: the Rotterdam study. *Invest Ophthalmol Vis Sci* 2005;46:1182-1187.
185. Tan AG, Mitchell P, Burlutsky G, et al. Retinal vessel caliber and the long-term incidence of age-related cataract: the Blue Mountains Eye Study. *Ophthalmology* 2008;115:1693-1698, 1698 e1691.
186. Kalyani PS, Fawzi AA, Gangaputra S, et al. Retinal vessel caliber among people with acquired immunodeficiency syndrome: relationships with visual function. *Am J Ophthalmol* 153:428-433 e421.
187. Liew G, Kaushik S, Rochtchina E, Tan AG, Mitchell P, Wang JJ. Retinal vessel signs and 10-year incident age-related maculopathy: the Blue Mountains Eye Study. *Ophthalmology* 2006;113:1481-1487.
188. Kagan A, Aureli E, Dobree J. A note on signs in the fundus oculi and arterial hypertension: conventional assessment and significance. *Bull World Health Organ* 1966;34:955-960.
189. Dimmitt SB, West JN, Eames SM, Gibson JM, Gosling P, Littler WA. Usefulness of ophthalmoscopy in mild to moderate hypertension. *Lancet* 1989;1:1103-1106.

190. Jeganathan VS, Kawasaki R, Wang JJ, et al. Retinal vascular caliber and age-related macular degeneration: the Singapore Malay Eye Study. *Am J Ophthalmol* 2008;146:954-959 e951.
191. Ikram MK, van Leeuwen R, Vingerling JR, Hofman A, de Jong PT. Retinal vessel diameters and the risk of incident age-related macular disease: the Rotterdam Study. *Ophthalmology* 2005;112:548-552.
192. Nagaoka T, Yoshida A. Noninvasive evaluation of wall shear stress on retinal microcirculation in humans. *Invest Ophthalmol Vis Sci* 2006;47:1113-1119.
193. Nagaoka T, Sakamoto T, Mori F, Sato E, Yoshida A. The effect of nitric oxide on retinal blood flow during hypoxia in cats. *Invest Ophthalmol Vis Sci* 2002;43:3037-3044.
194. Rassam SM, Patel V, Kohner EM. The effect of experimental hypertension on retinal vascular autoregulation in humans: a mechanism for the progression of diabetic retinopathy. *Exp Physiol* 1995;80:53-68.
195. Grunwald JE, Riva CE, Stone RA, Keates EU, Petrig BL. Retinal autoregulation in open-angle glaucoma. *Ophthalmology* 1984;91:1690-1694.
196. Frederiksen CA, Jeppesen P, Knudsen ST, Poulsen PL, Mogensen CE, Bek T. The blood pressure-induced diameter response of retinal arterioles decreases with increasing diabetic maculopathy. *Graefes Arch Clin Exp Ophthalmol* 2006;244:1255-1261.
197. Roufail E, Stringer M, Rees S. Nitric oxide synthase immunoreactivity and NADPH diaphorase staining are co-localised in neurons closely associated with the vasculature in rat and human retina. *Brain Res* 1995;684:36-46.
198. Tamai K, Matsubara A, Tomida K, et al. Lipid hydroperoxide stimulates leukocyte-endothelium interaction in the retinal microcirculation. *Exp Eye Res* 2002;75:69-75.
199. Surmacz E. Obesity hormone leptin: a new target in breast cancer? *Breast Cancer Res* 2007;9:301.
200. Oren S, Grossman E, Frohlich ED. Arterial and venous compliance in obese and nonobese subjects. *Am J Cardiol* 1996;77:665-667.
201. Vecchione C, Maffei A, Colella S, et al. Leptin effect on endothelial nitric oxide is mediated through Akt-endothelial nitric oxide synthase phosphorylation pathway. *Diabetes* 2002;51:168-173.
202. Sng CC, Sabanayagam C, Lamoureux EL, et al. Fractal analysis of the retinal vasculature and chronic kidney disease. *Nephrol Dial Transplant* 2010;25:2252-2258.
203. Boone MI, Farber ME, Jovanovic-Peterson L, Peterson CM. Increased retinal vascular tortuosity in gestational diabetes mellitus. *Ophthalmology* 1989;96:251-254.
204. Trucco E, Azegrouz H, Dhillon B. Modeling the tortuosity of retinal vessels: does caliber play a role? *IEEE Trans Biomed Eng* 2010;57:2239-2247.
205. Lehmann MV, Schmieder RE. Remodeling of retinal small arteries in hypertension. *Am J Hypertens* 2011;24:1267-1273.
206. Schmieder RE. Hypertensive retinopathy: a window to vascular remodeling in arterial hypertension. *Hypertension* 2008;51:43-44.
207. Adair L, Dahly D. Developmental determinants of blood pressure in adults. *Annu Rev Nutr* 2005;25:407-434.
208. Toprak A, Wang H, Chen W, Paul T, Srinivasan S, Berenson G. Relation of childhood risk factors to left ventricular hypertrophy (eccentric or concentric) in relatively young adulthood (from the Bogalusa Heart Study). *Am J Cardiol* 2008;101:1621-1625.
209. Rademacher ER, Jacobs DR, Jr., Moran A, Steinberger J, Prineas RJ, Sinaiko A. Relation of blood pressure and body mass index during childhood to cardiovascular risk factor levels in young adults. *J Hypertens* 2009;27:1766-1774.

210. Liang L, Mi J, Zhang MM, Wang YF, Wang TY. [Study on the impact of the choice of diastolic Korotkoff phase in childhood on prediction to adult hypertension]. *Zhonghua Liu Xing Bing Xue Za Zhi* 2008;29:110-115.
211. Barker DJ, Forsen T, Eriksson JG, Osmond C. Growth and living conditions in childhood and hypertension in adult life: a longitudinal study. *J Hypertens* 2002;20:1951-1956.
212. Raitakari OT, Juonala M, Kahonen M, et al. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. *JAMA* 2003;290:2277-2283.
213. Lain KY, Roberts JM. Contemporary concepts of the pathogenesis and management of preeclampsia. *JAMA* 2002;287:3183-3186.
214. Seely EW, Ecker J. Clinical practice. Chronic hypertension in pregnancy. *N Engl J Med* 2011;365:439-446.
215. Bakker R, Steegers EA, Hofman A, Jaddoe VW. Blood pressure in different gestational trimesters, fetal growth, and the risk of adverse birth outcomes: the generation R study. *Am J Epidemiol* 2011;174:797-806.
216. Khan KS, Wojdyla D, Say L, Gulmezoglu AM, Van Look PF. WHO analysis of causes of maternal death: a systematic review. *Lancet* 2006;367:1066-1074.
217. Roberts CL, Bell JC, Ford JB, Hadfield RM, Algert CS, Morris JM. The accuracy of reporting of the hypertensive disorders of pregnancy in population health data. *Hypertens Pregnancy* 2008;27:285-297.
218. Irgens HU, Reisaeter L, Irgens LM, Lie RT. Long term mortality of mothers and fathers after pre-eclampsia: population based cohort study. *BMJ* 2001;323:1213-1217.
219. Cnossen JS, Vollebregt KC, de Vrieze N, et al. Accuracy of mean arterial pressure and blood pressure measurements in predicting pre-eclampsia: systematic review and meta-analysis. *BMJ* 2008;336:1117-1120.
220. Gaillard R, Bakker R, Willemsen SP, Hofman A, Steegers EA, Jaddoe VW. Blood pressure tracking during pregnancy and the risk of gestational hypertensive disorders: The Generation R Study. *Eur Heart J* 2011.
221. Gallery ED. Pregnancy-associated hypertension: interrelationships of volume and blood pressure changes. *Clin Exp Hypertens B* 1982;1:39-47.
222. Daniels SR. Complications of obesity in children and adolescents. *Int J Obes (Lond)* 2009;33 Suppl 1:S60-65.
223. Mamun AA, Hayatbakhsh MR, O'Callaghan M, Williams G, Najman J. Early overweight and pubertal maturation--pathways of association with young adults' overweight: a longitudinal study. *Int J Obes (Lond)* 2009;33:14-20.
224. Savino A, Pelliccia P, Chiarelli F, Mohn A. Obesity-related renal injury in childhood. *Horm Res Paediatr* 2010;73:303-311.
225. Thomsen SF, Ulrik CS, Kyvik KO, et al. Association between obesity and asthma in a twin cohort. *Allergy* 2007;62:1199-1204.
226. Wong TY, Klein R, Couper DJ, et al. Retinal microvascular abnormalities and incident stroke: the Atherosclerosis Risk in Communities Study. *Lancet* 2001;358:1134-1140.
227. Tracy RE, Newman WP, 3rd, Wattigney WA, Berenson GS. Risk factors and atherosclerosis in youth autopsy findings of the Bogalusa Heart Study. *Am J Med Sci* 1995;310 Suppl 1:S37-41.
228. Zieske AW, Malcom GT, Strong JP. Natural history and risk factors of atherosclerosis in children and youth: the PDAY study. *Pediatr Pathol Mol Med* 2002;21:213-237.

229. Davis PH, Dawson JD, Riley WA, Lauer RM. Carotid intimal-medial thickness is related to cardiovascular risk factors measured from childhood through middle age: The Muscatine Study. *Circulation* 2001;104:2815-2819.
230. Li S, Chen W, Srinivasan SR, et al. Childhood cardiovascular risk factors and carotid vascular changes in adulthood: the Bogalusa Heart Study. *JAMA* 2003;290:2271-2276.
231. Catalano PM. Obesity, insulin resistance, and pregnancy outcome. *Reproduction* 2010;140:365-371.
232. Dixit A, Girling JC. Obesity and pregnancy. *J Obstet Gynaecol* 2008;28:14-23.
233. Melzer K, Schutz Y. Pre-pregnancy and pregnancy predictors of obesity. *Int J Obes (Lond)* 2010;34 Suppl 2:S44-52.
234. Yogev Y, Catalano PM. Pregnancy and obesity. *Obstet Gynecol Clin North Am* 2009;36:285-300, viii.
235. Smith SA, Hulsey T, Goodnight W. Effects of obesity on pregnancy. *J Obstet Gynecol Neonatal Nurs* 2008;37:176-184.
236. Saw SM, Shankar A, Tan SB, et al. A cohort study of incident myopia in Singaporean children. *Invest Ophthalmol Vis Sci* 2006;47:1839-1844.
237. Chee CY, Chong YS, Ng TP, Lee DT, Tan LK, Fones CS. The association between maternal depression and frequent non-routine visits to the infant's doctor--a cohort study. *J Affect Disord* 2008;107:247-253.
238. Nicholson A, Kuper H, Hemingway H. Depression as an aetiologic and prognostic factor in coronary heart disease: a meta-analysis of 6362 events among 146 538 participants in 54 observational studies. *Eur Heart J* 2006;27:2763-2774.
239. Carney RM, Freedland KE. Depression, mortality, and medical morbidity in patients with coronary heart disease. *Biol Psychiatry* 2003;54:241-247.
240. Miller MA, Cappuccio FP. Inflammation, sleep, obesity and cardiovascular disease. *Curr Vasc Pharmacol* 2007;5:93-102.
241. Motivala SJ. Sleep and Inflammation: Psychoneuroimmunology in the Context of Cardiovascular Disease. *Ann Behav Med* 2011.
242. Fiedorowicz JG, He J, Merikangas KR. The association between mood and anxiety disorders with vascular diseases and risk factors in a nationally representative sample. *J Psychosom Res* 2011;70:145-154.
243. Qiu C, Sanchez SE, Lam N, Garcia P, Williams MA. Associations of depression and depressive symptoms with preeclampsia: results from a Peruvian case-control study. *BMC Womens Health* 2007;7:15.
244. Rodie VA, Freeman DJ, Sattar N, Greer IA. Pre-eclampsia and cardiovascular disease: metabolic syndrome of pregnancy? *Atherosclerosis* 2004;175:189-202.
245. Gold PW, Chrousos GP. The endocrinology of melancholic and atypical depression: relation to neurocircuitry and somatic consequences. *Proc Assoc Am Physicians* 1999;111:22-34.
246. Kop WJ, Synowski SJ, Gottlieb SS. Depression in heart failure: biobehavioral mechanisms. *Heart Fail Clin* 2011;7:23-38.
247. Bennett HA, Einarson A, Taddio A, Koren G, Einarson TR. Prevalence of depression during pregnancy: systematic review. *Obstet Gynecol* 2004;103:698-709.
248. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics* 2004;114:555-576.
249. Ogden CL, Kuczmarski RJ, Flegal KM, et al. Centers for Disease Control and Prevention 2000 growth charts for the United States: improvements to the 1977 National Center for Health Statistics version. *Pediatrics* 2002;109:45-60.

250. Must A, Dallal GE, Dietz WH. Reference data for obesity: 85th and 95th percentiles of body mass index (wt/ht2) and triceps skinfold thickness. *Am J Clin Nutr* 1991;53:839-846.
251. Kohn MA, Vosti CL, Lezotte D, Jones RH. Optimal gestational age and birth-weight cutoffs to predict neonatal morbidity. *Med Decis Making* 2000;20:369-376.
252. Low W, Dirani M, Gazzard G, et al. Family history, near work, outdoor activity, and myopia in Singapore Chinese preschool children. *Br J Ophthalmol* 2010;94:1012-1016.
253. Dirani M, Zhou B, Hornbeak D, et al. Prevalence and causes of decreased visual acuity in Singaporean Chinese preschoolers. *Br J Ophthalmol* 2010;94:1561-1565.
254. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabetic medicine : a journal of the British Diabetic Association* 1998;15:539-553.
255. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. *Lancet* 2005;365:785-799.
256. Papageorghiou AT. Predicting and preventing pre-eclampsia-where to next? *Ultrasound Obstet Gynecol* 2008;31:367-370.
257. Farag K, Hassan I, Ledger WL. Prediction of preeclampsia: can it be achieved? *Obstet Gynecol Surv* 2004;59:464-482; quiz 485.
258. Jafar TH, Chaturvedi N, Pappas G. Prevalence of overweight and obesity and their association with hypertension and diabetes mellitus in an Indo-Asian population. *CMAJ* 2006;175:1071-1077.
259. Medicine Io. Nutritional status and weight gain. In: IoM, editors. Nutrition during pregnancy. *Washington, DC: National Academy Press* 1990;227-233.
260. Cox JL, Holden JM, Sagovsky R. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. *Br J Psychiatry* 1987;150:782-786.
261. Kendall PC, Finch AJ, Jr., Auerbach SM, Hooke JF, Mikulka PJ. The State-Trait Anxiety Inventory: a systematic evaluation. *J Consult Clin Psychol* 1976;44:406-412.
262. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res* 1989;28:193-213.
263. Gibson J, McKenzie-McHarg K, Shakespeare J, Price J, Gray R. A systematic review of studies validating the Edinburgh Postnatal Depression Scale in antepartum and postpartum women. *Acta Psychiatr Scand* 2009;119:350-364.
264. Teixeira JM, Fisk NM, Glover V. Association between maternal anxiety in pregnancy and increased uterine artery resistance index: cohort based study. *BMJ* 1999;318:153-157.
265. Bengtsson B, Krakau CE. Correction of optic disc measurements on fundus photographs. *Graefes Arch Clin Exp Ophthalmol* 1992;230:24-28.
266. Gaillard R, Bakker R, Willemsen SP, Hofman A, Steegers EA, Jaddoe VW. Blood pressure tracking during pregnancy and the risk of gestational hypertensive disorders: The Generation R Study. *Eur Heart J* 32:3088-3097.
267. Walsh CA, Baxi LV. Mean arterial pressure and prediction of pre-eclampsia. *BMJ* 2008;336:1079-1080.
268. Cifkova R. Can blood pressure in the first trimester predict the development of gestational hypertensive disorders? *Eur Heart J* 32:3067-3069.
269. Waugh J, Perry IJ, Halligan AW, et al. Birth weight and 24-hour ambulatory blood pressure in nonproteinuric hypertensive pregnancy. *Am J Obstet Gynecol* 2000;183:633-637.
270. Duvekot JJ, Peeters LL. Maternal cardiovascular hemodynamic adaptation to pregnancy. *Obstet Gynecol Surv* 1994;49:S1-14.

271. Hughes AD, Wong TY, Witt N, et al. Determinants of retinal microvascular architecture in normal subjects. *Microcirculation* 2009;16:159-166.
272. Wong TY, Hubbard LD, Klein R, et al. Retinal microvascular abnormalities and blood pressure in older people: the Cardiovascular Health Study. *Br J Ophthalmol* 2002;86:1007-1013.
273. Skalina ME, Annable WL, Kliegman RM, Fanaroff AA. Hypertensive retinopathy in the newborn infant. *J Pediatr* 1983;103:781-786.
274. Daniels SR, Lipman MJ, Burke MJ, Loggie JM. The prevalence of retinal vascular abnormalities in children and adolescents with essential hypertension. *Am J Ophthalmol* 1991;111:205-208.
275. Mitchell P, Wang JJ, Wong TY, Smith W, Klein R, Leeder SR. Retinal microvascular signs and risk of stroke and stroke mortality. *Neurology* 2005;65:1005-1009.
276. Ikram MK, de Jong FJ, Bos MJ, et al. Retinal vessel diameters and risk of stroke: the Rotterdam Study. *Neurology* 2006;66:1339-1343.
277. Wang JJ, Liew G, Wong TY, et al. Retinal vascular calibre and the risk of coronary heart disease-related death. *Heart* 2006;92:1583-1587.
278. Klein BE, Klein R, McBride PE, et al. Cardiovascular disease, mortality, and retinal microvascular characteristics in type 1 diabetes: Wisconsin epidemiologic study of diabetic retinopathy. *Arch Intern Med* 2004;164:1917-1924.
279. Nguyen TT, Wang JJ, Wong TY. Retinal vascular changes in pre-diabetes and prehypertension: new findings and their research and clinical implications. *Diabetes Care* 2007;30:2708-2715.
280. Witt N, Wong TY, Hughes AD, et al. Abnormalities of retinal microvascular structure and risk of mortality from ischemic heart disease and stroke. *Hypertension* 2006;47:975-981.
281. Murray CD. The Physiological Principle of Minimum Work: I. The Vascular System and the Cost of Blood Volume. *Proc Natl Acad Sci U S A* 1926;12:207-214.
282. Ingelfinger JR. Pediatric antecedents of adult cardiovascular disease--awareness and intervention. *N Engl J Med* 2004;350:2123-2126.
283. Paul TK, Srinivasan SR, Wei C, et al. Cardiovascular risk profile of asymptomatic healthy young adults with increased femoral artery intima-media thickness: The Bogalusa Heart Study. *Am J Med Sci* 2005;330:105-110.
284. Nagaoka T, Mori F, Yoshida A. Retinal artery response to acute systemic blood pressure increase during cold pressor test in humans. *Invest Ophthalmol Vis Sci* 2002;43:1941-1945.
285. Mi J, Law C, Zhang KL, Osmond C, Stein C, Barker D. Effects of infant birthweight and maternal body mass index in pregnancy on components of the insulin resistance syndrome in China. *Ann Intern Med* 2000;132:253-260.
286. Forsen T, Eriksson JG, Tuomilehto J, Teramo K, Osmond C, Barker DJ. Mother's weight in pregnancy and coronary heart disease in a cohort of Finnish men: follow up study. *BMJ* 1997;315:837-840.
287. Guelinckx I, Devlieger R, Beckers K, Vansant G. Maternal obesity: pregnancy complications, gestational weight gain and nutrition. *Obes Rev* 2008;9:140-150.
288. Han JC, Lawlor DA, Kimm SY. Childhood obesity. *Lancet* 2010;375:1737-1748.
289. Taylor ED, Theim KR, Mirch MC, et al. Orthopedic complications of overweight in children and adolescents. *Pediatrics* 2006;117:2167-2174.
290. Cheung N, Saw SM, Islam FM, et al. BMI and retinal vascular caliber in children. *Obesity (Silver Spring)* 2007;15:209-215.

291. Li LJ, Cheung CY, Chia A, et al. The relationship of body fatness indices and retinal vascular caliber in children. *Int J Pediatr Obes* 2011;6:267-274.
292. Kip KE, Marroquin OC, Kelley DE, et al. Clinical importance of obesity versus the metabolic syndrome in cardiovascular risk in women: a report from the Women's Ischemia Syndrome Evaluation (WISE) study. *Circulation* 2004;109:706-713.
293. Raj M. Obesity and cardiovascular risk in children and adolescents. *Indian J Endocrinol Metab* 16:13-19.
294. Mei Z, Grummer-Strawn LM, Wang J, et al. Do skinfold measurements provide additional information to body mass index in the assessment of body fatness among children and adolescents? *Pediatrics* 2007;119:e1306-1313.
295. Tomoda S, Tamura T, Sudo Y, Ogita S. Effects of obesity on pregnant women: maternal hemodynamic change. *Am J Perinatol* 1996;13:73-78.
296. Galtier-Dereure F, Boegner C, Bringer J. Obesity and pregnancy: complications and cost. *Am J Clin Nutr* 2000;71:1242S-1248S.
297. Frederick IO, Rudra CB, Miller RS, Foster JC, Williams MA. Adult weight change, weight cycling, and prepregnancy obesity in relation to risk of preeclampsia. *Epidemiology* 2006;17:428-434.
298. Naeye RL. Maternal body weight and pregnancy outcome. *Am J Clin Nutr* 1990;52:273-279.
299. Johnson SR, Kolberg BH, Varner MW, Railsback LD. Maternal obesity and pregnancy. *Surg Gynecol Obstet* 1987;164:431-437.
300. Garbaciak JA, Jr., Richter M, Miller S, Barton JJ. Maternal weight and pregnancy complications. *Am J Obstet Gynecol* 1985;152:238-245.
301. Abrams B, Parker J. Overweight and pregnancy complications. *Int J Obes* 1988;12:293-303.
302. Edwards LE, Hellerstedt WL, Alton IR, Story M, Himes JH. Pregnancy complications and birth outcomes in obese and normal-weight women: effects of gestational weight change. *Obstet Gynecol* 1996;87:389-394.
303. Galtier-Dereure F, Montpeyroux F, Boulot P, Bringer J, Jaffiol C. Weight excess before pregnancy: complications and cost. *Int J Obes Relat Metab Disord* 1995;19:443-448.
304. Larsen TB, Sorensen HT, Gislum M, Johnsen SP. Maternal smoking, obesity, and risk of venous thromboembolism during pregnancy and the puerperium: a population-based nested case-control study. *Thromb Res* 2007;120:505-509.
305. Yamakawa K, Bhutto IA, Lu Z, Watanabe Y, Amemiya T. Retinal vascular changes in rats with inherited hypercholesterolemia--corrosion cast demonstration. *Curr Eye Res* 2001;22:258-265.
306. Tomita Y, Kubis N, Calando Y, et al. Long-term in vivo investigation of mouse cerebral microcirculation by fluorescence confocal microscopy in the area of focal ischemia. *J Cereb Blood Flow Metab* 2005;25:858-867.
307. Ko GT, Chan JC, Cockram CS, Woo J. Prediction of hypertension, diabetes, dyslipidaemia or albuminuria using simple anthropometric indexes in Hong Kong Chinese. *Int J Obes Relat Metab Disord* 1999;23:1136-1142.
308. He J, Klag MJ, Whelton PK, Chen JY, Qian MC, He GQ. Body mass and blood pressure in a lean population in southwestern China. *Am J Epidemiol* 1994;139:380-389.
309. Park YW, Allison DB, Heymsfield SB, Gallagher D. Larger amounts of visceral adipose tissue in Asian Americans. *Obes Res* 2001;9:381-387.

310. Rattarasarn C, Soonthornpan S, Leelawattana R, Setasuban W. Decreased insulin secretion but not insulin sensitivity in normal glucose tolerant Thai subjects. *Diabetes Care* 2006;29:742-743.
311. Fukushima M, Suzuki H, Seino Y. Insulin secretion capacity in the development from normal glucose tolerance to type 2 diabetes. *Diabetes Res Clin Pract* 2004;66 Suppl 1:S37-43.
312. Kolodjaschna J, Berisha F, Lung S, et al. LPS-induced microvascular leukocytosis can be assessed by blue-field entoptic phenomenon. *Am J Physiol Heart Circ Physiol* 2004;287:H691-694.
313. Procopio C, Andreozzi F, Laratta E, et al. Leptin-stimulated endothelial nitric-oxide synthase via an adenosine 5'-monophosphate-activated protein kinase/Akt signaling pathway is attenuated by interaction with C-reactive protein. *Endocrinology* 2009;150:3584-3593.
314. Guo SS, Huang C, Maynard LM, et al. Body mass index during childhood, adolescence and young adulthood in relation to adult overweight and adiposity: the Fels Longitudinal Study. *Int J Obes Relat Metab Disord* 2000;24:1628-1635.
315. Power C, Jefferis BJ, Manor O. Childhood cognition and risk factors for cardiovascular disease in midadulthood: the 1958 British Birth Cohort Study. *Am J Public Health* 2010;100:129-136.
316. Starc G, Strel J. Tracking excess weight and obesity from childhood to young adulthood: a 12-year prospective cohort study in Slovenia. *Public Health Nutr* 2010;1-7.
317. Srinivasan SR, Myers L, Berenson GS. Predictability of childhood adiposity and insulin for developing insulin resistance syndrome (syndrome X) in young adulthood: the Bogalusa Heart Study. *Diabetes* 2002;51:204-209.
318. Alemzadeh R, Kichler J, Babar G, Calhoun M. Hypovitaminosis D in obese children and adolescents: relationship with adiposity, insulin sensitivity, ethnicity, and season. *Metabolism* 2008;57:183-191.
319. Bjorge T, Engeland A, Tverdal A, Smith GD. Body mass index in adolescence in relation to cause-specific mortality: a follow-up of 230,000 Norwegian adolescents. *Am J Epidemiol* 2008;168:30-37.
320. Fan DS, Lai C, Lau HH, Cheung EY, Lam DS. Change in vision disorders among Hong Kong preschoolers in ten years. *Clin Experiment Ophthalmol* 2010.
321. Lai YH, Hsu HT, Wang HZ, Chang SJ, Wu WC. The visual status of children ages 3 to 6 years in the vision screening program in Taiwan. *J AAPOS* 2009;13:58-62.
322. Ikuno Y. Pathogenesis and treatment of myopic foveoschisis. *Nippon Ganka Gakkai Zasshi* 2006;110:855-863.
323. Ikuno Y, Sayanagi K, Soga K, et al. Lacquer crack formation and choroidal neovascularization in pathologic myopia. *Retina* 2008;28:1124-1131.
324. Ikuno Y, Tano Y. Early macular holes with retinoschisis in highly myopic eyes. *Am J Ophthalmol* 2003;136:741-744.
325. Wong TY, Foster PJ, Johnson GJ, Seah SK. Refractive errors, axial ocular dimensions, and age-related cataracts: the Tanjong Pagar survey. *Invest Ophthalmol Vis Sci* 2003;44:1479-1485.
326. Wong TY, Klein BE, Klein R, Knudtson M, Lee KE. Refractive errors, intraocular pressure, and glaucoma in a white population. *Ophthalmology* 2003;110:211-217.
327. Shimada N, Ohno-Matsui K, Harino S, et al. Reduction of retinal blood flow in high myopia. *Graefes Arch Clin Exp Ophthalmol* 2004;242:284-288.
328. Akyol N, Kukner AS, Ozdemir T, Esmerligil S. Choroidal and retinal blood flow changes in degenerative myopia. *Can J Ophthalmol* 1996;31:113-119.

329. Nemeth J, Michelson G, Harazny J. Retinal microcirculation correlates with ocular wall thickness, axial eye length, and refraction in glaucoma patients. *J Glaucoma* 2001;10:390-395.
330. Dimitrova G, Tamaki Y, Kato S, Nagahara M. Retrobulbar circulation in myopic patients with or without myopic choroidal neovascularisation. *Br J Ophthalmol* 2002;86:771-773.
331. Lim LS, Cheung CY, Lin X, Mitchell P, Wong TY, Saw SM. Influence of refractive error and axial length on retinal vessel geometric characteristics. *Invest Ophthalmol Vis Sci* 2010.
332. Patton N, Maini R, MacGillivary T, Aslam TM, Deary IJ, Dhillon B. Effect of axial length on retinal vascular network geometry. *Am J Ophthalmol* 2005;140:648-653.
333. Pierro L, Camesasca FI, Mischi M, Brancato R. Peripheral retinal changes and axial myopia. *Retina* 1992;12:12-17.
334. Islam FM, Nguyen TT, Wang JJ, et al. Quantitative retinal vascular calibre changes in diabetes and retinopathy: the Singapore Malay eye study. *Eye (Lond)* 2009;23:1719-1724.
335. Laurence S Lim XY, Seang-Mei Saw, Anqi Qiu. Variation in eye volume, surface area, and shape with reractive error in young children by magnetic resonance imaging analysis. *submmitted in IOVS* 2010.
336. Saw SM, Gazzard G, Shih-Yen EC, Chua WH. Myopia and associated pathological complications. *Ophthalmic Physiol Opt* 2005;25:381-391.
337. Lim LS, Lamoureux E, Saw SM, Tay WT, Mitchell P, Wong TY. Are myopic eyes less likely to have diabetic retinopathy? *Ophthalmology* 117:524-530.
338. Longanesi L, Cavallini GM, Toni R. Quantitative clinical anatomy of the human cornea in vivo. A morphometric study by ultrasonic pachymetry and computer-assisted topographic videokeratoscopy. *Acta Anat (Basel)* 1996;157:73-79.
339. Shimmyo M. Intraocular pressure, Goldmann applanation tension, corneal thickness, and corneal curvature in Caucasians, Asians, Hispanics, and African Americans. *Am J Ophthalmol* 2004;137:1170.
340. Bohm AG. [The effect of central corneal thickness on tonometry]. *Klin Monbl Augenheilkd* 2011;228:114-117.
341. Lesk MR, Hafez AS, Descovich D. Relationship between central corneal thickness and changes of optic nerve head topography and blood flow after intraocular pressure reduction in open-angle glaucoma and ocular hypertension. *Arch Ophthalmol* 2006;124:1568-1572.
342. Doughty MJ, Zaman ML. Human corneal thickness and its impact on intraocular pressure measures: a review and meta-analysis approach. *Surv Ophthalmol* 2000;44:367-408.
343. Shin DH, Tsai CS, Parrow KA, Kim C, Wan JY, Shi DX. Intraocular pressure-dependent retinal vascular change in adult chronic open-angle glaucoma patients. *Ophthalmology* 1991;98:1087-1092.
344. Nguyen TT, Wong TY, Islam FM, et al. Evidence of early retinal microvascular changes in patients with type 2 diabetes and depression. *Psychosom Med* 2010;72:535-538.
345. Kim DH, Newman AB, Hajjar I, et al. Retinal microvascular signs and functional loss in older persons: the cardiovascular health study. *Stroke* 2011;42:1589-1595.
346. Ikram MK, Luijendijk HJ, Hofman A, et al. Retinal vascular calibers and risk of late-life depression: The Rotterdam Study. *Am J Geriatr Psychiatry* 2010;18:452-455.
347. Leung BM, Kaplan BJ. Perinatal depression: prevalence, risks, and the nutrition link--a review of the literature. *J Am Diet Assoc* 2009;109:1566-1575.
348. Granger DN, Senchenkova E. Inflammation and the Microcirculation. 2010.

349. Chen H, Yiu KH, Tse HF. Relationships between vascular dysfunction, circulating endothelial progenitor cells, and psychological status in healthy subjects. *Depress Anxiety* 2011;28:719-727.
350. MacRitchie AN, Jun SS, Chen Z, et al. Estrogen upregulates endothelial nitric oxide synthase gene expression in fetal pulmonary artery endothelium. *Circ Res* 1997;81:355-362.
351. Kasasbeh E, Chi DS, Krishnaswamy G. Inflammatory aspects of sleep apnea and their cardiovascular consequences. *South Med J* 2006;99:58-67; quiz 68-59, 81.
352. Resnick HE, Howard BV. Diabetes and cardiovascular disease. *Annu Rev Med* 2002;53:245-267.
353. Clearfield MB. C-reactive protein: a new risk assessment tool for cardiovascular disease. *J Am Osteopath Assoc* 2005;105:409-416.
354. Teramoto S, Yamamoto H, Ouchi Y. Increased C-reactive protein and increased plasma interleukin-6 may synergistically affect the progression of coronary atherosclerosis in obstructive sleep apnea syndrome. *Circulation* 2003;107:E40-40.
355. Colloby SJ, Firbank MJ, He J, et al. Regional cerebral blood flow in late-life depression: arterial spin labelling magnetic resonance study. *Br J Psychiatry* 2012;200:150-155.
356. Pagani M, Gardner A, Salmaso D, et al. Principal component and volume of interest analyses in depressed patients imaged by 99mTc-HMPAO SPET: a methodological comparison. *Eur J Nucl Med Mol Imaging* 2004;31:995-1004.
357. Smith GS, Kramer E, Ma Y, et al. The functional neuroanatomy of geriatric depression. *Int J Geriatr Psychiatry* 2009;24:798-808.
358. Huang A, Sun D, Koller A, Kaley G. 17beta-estradiol restores endothelial nitric oxide release to shear stress in arterioles of male hypertensive rats. *Circulation* 2000;101:94-100.
359. Ozanne SE, Hales CN. Early programming of glucose-insulin metabolism. *Trends in endocrinology and metabolism: TEM* 2002;13:368-373.
360. Levitt NS, Lambert EV. The foetal origins of the metabolic syndrome--a South African perspective. *Cardiovascular journal of South Africa : official journal for Southern Africa Cardiac Society [and] South African Society of Cardiac Practitioners* 2002;13:179-180.
361. Ehrenberg HM, Huston-Presley L, Catalano PM. The influence of obesity and gestational diabetes mellitus on accretion and the distribution of adipose tissue in pregnancy. *Am J Obstet Gynecol* 2003;189:944-948.
362. Kinoshita T, Itoh M. Longitudinal variance of fat mass deposition during pregnancy evaluated by ultrasonography: the ratio of visceral fat to subcutaneous fat in the abdomen. *Gynecol Obstet Invest* 2006;61:115-118.
363. Yu SM, Nagey DA. Validity of self-reported pregravid weight. *Ann Epidemiol* 1992;2:715-721.
364. Saldana TM, Siega-Riz AM, Adair LS. Effect of macronutrient intake on the development of glucose intolerance during pregnancy. *Am J Clin Nutr* 2004;79:479-486.
365. Lederman SA, Paxton A. Maternal reporting of prepregnancy weight and birth outcome: consistency and completeness compared with the clinical record. *Matern Child Health J* 1998;2:123-126.

Publications during Ph.D:

1. **Ling-Jun Li**, CYL Cheung, MK Ikram, P Gluckman, K Kwek, YS Chong, MJ Meaney, TY Wong, SM Saw. Blood Pressure and Retinal Microvascular Characterisitcs During Pregnancy : Growing Up in Singapore Towards Healthy Outcomes (GUSTO) Study. *Hypertension*. 2012;60(1) :223-30.
2. **Ling-Jun Li**, MK Ikram, CYL Cheung, YS Lee, LJ Lee, P Gluckman, K Kwek, YS Chong, TY Wong, SM Saw. Effect of Maternal Body Mass Index on the Retinal Microvasculature in Pregnancy. Already accepted by *Obstetrics & Gynecology*. 2012.
3. **Ling-Jun Li,** CYL Cheung, G Gazzard, P Mitchell, TY Wong, SM Saw. Relationship of ocular biometry and retinal vascular caliber in preschoolers. *IOVS*. 2011; 52(13):9561-6.
4. **Ling-Jun Li,** CYL Cheung, A Chia, P Selvaraj, XY Lin, P Mitchell, TY Wong, SM Saw. The relationship of body fatness indices and retinal vascular caliber in children. Int J Pediatr Obes. 2011 Aug;6(3-4):267-74.
5. **Ling-Jun Li,** CYL Cheung, Y Liu, A Chia, P Selvaraj, XY Lin, YM Chan, R Varma, P Mitchell, TY Wong, SM Saw. Influence of blood pressure on retinal vascular caliber in young children. *Ophthalmology*. 2011;118(7):1459-65.
6. **Ling-Jun Li**, MK Ikram, L Broekman, CYL Cheung, H Chen, JJ Gooley, SE Soh, P Gluckman, K Kwek, YS Chong, MJ Meaney, TY Wong, SM Saw. Antenatal Mental Health and Changes of Retinal Microvasculature during Pregnancy. Submitted to *TVST*. 2012.

Book Chapter :

Ling-Jun Li, Tien-Yin Wong, Seang-Mei Saw. Children Obesity, Body Fatness Indices and Retinal Vasculature. *Handbook of Diet, Nutrition and The Eye*. King's College. 2012.

Awards during Ph.D

1. Brozne Medal of Singapore Young Investigator Award by the Singapore Health & Biomedical Congress (SHBC) 2012
2. March 2012 Nominated for National University of Singapore *President Graduate Fellowship*
3. June 2012 28th Singapore-Malaysia Ophthalmology Symposium *Merit Poster Award*
4. Aug 2011 6th Singapore Pubic Health & Occupational Medicine Conference *Oral Presentation Merit Award*
5. May 2011 ARVO (The Association for Research in Vision and Ophthalmology, Fort Lauderdale, US) *Travel grant award*
6. Aug 2009 National University Singapore Research Scholarship

Personal Contributions in This Study

I have taken part in the data collection, data entry, and clinical ground work such as recruitments, questionnaires interview and physical measurements. After qualified for retinal photography examination and grading, all fundus examination and retinal imaging assessments were done by me for the GUSTO/GUSTO IVF cohort. Furthermore, I also took part in the study design for retinal follow-up study in pregnant women and children, such as drafting part of the grant application. All data analyses were done by me and all manuscripts were written by me as the first-author. In the meantime, I was also invited to review original articles or systemic reviews/meta-analysis for *Ophthalmology*, *Plos One* and *IOVS*.

STUDY ID: ☐☐☐☐ _ ☐ _ ☐☐

(Home ID) (Fam ID) (Child ID)

[sno]

INTERVIEWER ID: ☐☐☐

[invest2]

DATE OF INTERVIEW: _____

[date2] (DD – MM – YYYY)

Sticky Label with following information:

1. Name: [name]

2. BC number: [bcno]

2. Age: [agemth]

A Study on Strabismus, Amblyopia and Refractive Error in Singapore Preschoolers

新加坡学前孩童于折射误差，弱视和斜视方面的研究

Clinic Questionnaire

诊所问卷

(1 Interview / Child)
(1 采访 / 儿童)

Remarks 备注:

_____

_____

_____

_____

START TIME ☐☐:☐☐ AM/PM

OBTAIN INFORMED CONSENT FROM A PARENT OR LEGAL GUARDIAN FOR EACH CHILD BEFORE PROCEEDING WITH INTERVIEW FOR THAT CHILD.

在进行这项访问之前，必须得到每个孩童的父母或合法监护人的同意。

While your child is participating in the eye exam today I would like to ask you to fill in some questions to help researchers learn more about the eye health of children living in Singapore.

All the information you provide will be kept strictly confidential. You may choose not to answer any questions that you do not want to answer.

当您孩子今天在接受视力检查的同时，我想请您填写这份问卷以帮助研究人员了解更多有关新加坡孩童的视力健康问题。

您的作答将完全被保密。您可拒绝回答您不想回答的任何问题。

SECTION A: Healthcare Utilization

A 项：保健的使用

NAME 1

A1. What is your relationship to the children?

[childrel]

你和这孩子的关系是什么？

BIOLOGICAL MOTHER 亲生母亲.....1. ☐

BIOLOGICAL FATHER 亲生父亲.....2. ☐

STEP ADOPTIVE MOTHER 继母.....3. ☐

STEP ADOPTIVE FATHER 继父.....4. ☐

GRANDMOTHER 祖母.....5. ☐

GRANDFATHER 祖父.....6. ☐

AUNT 姑母.....7. ☐

UNCLE 姑父.....8. ☐

OTHER FEMALE RELATIVE 其他女性亲戚..... 9. ☐

(Specify) (说明)

_____

[femrel]

OTHER MALE RELATIVE 其他男性亲戚..... ..10. ☐

(Specify) (说明)

_____

[malerel]

OTHER FEMALE NON-RELATIVE

其他女性但不是亲戚 .....11. ☐

(Specify) (说明)

_____

[fnonrel]

OTHER MALE NON-RELATIVE

其他男性但不是亲戚 .....12. ☐

(Specify) (说明)

_____

RF 拒绝回答.....98. ☐

DK 不知道 .....99. ☐

[mnonrel]

A2. In what country was the child born?

您的孩子在哪里出世？

[country]

SINGAPORE 新加坡 ..... **(SKIP TO A 3)** ..... 1. ☐

MALAYSIA 马来西亚 .....**(GO TO A2 a)**..... 2. ☐

OTHER (SPECIFY)

其它 (说明) ..... **(GO TO A2 a)** ..... 3. ☐

[cntyspf]

Specify 请说明: _____

RF 88. ☐ DK 99. ☐

RF 拒绝回答 .....98. ☐

DK 不知道 .....99. ☐

A2. a When did your child move to Singapore?

您的孩子在哪一年移居新加坡？

[yrmove]

└┘└┘ .....YEAR 年

A3. Where was the child born?

[hospital]

您的孩子在哪里出生?

KKH 竹脚妇幼医院.....1. ☐

NUH 国立医院 .....2. ☐

SGH 中央医院 .....3. ☐

GLENEAGLES HOSPITAL 鹰阁医院 .....4. ☐

MOUNT AVERNIA.....5. ☐

THOMSON MEDICAL CENTER 康生 .....6. ☐

HOME 家里 .....7. ☐

GP CLINIC 普通医生诊所 .....8. ☐

OTHER 其它 .....9. ☐

[hosspf]

Specify 请说明: _____

RF 88. ☐ DK 99. ☐

RF 拒绝回答 .....98. ☐

DK 不知道 .....99. ☐

SECTION B: Pregnancy History

B 项：怀孕的经历

B1. How old were you when the child was born?

当您的孩子出生时，您多少岁？

[pregyear]

└┴┘ ..... YEARS OLD 岁

RF 拒绝回答 ..... 98. ☐

DK 不知道..... 99. ☐

B2. Was the child admitted to the neonatal intensive care unit? [neonate]

您的孩子有否进入新生儿的加护病房？

YES 是 ..... **(GO TO B2 a)** .....1. ☐

NO 没有..... **(SKIP TO B3)** .....0. ☐

RF 拒绝回答 ..... **(SKIP TO B3)** .....98. ☐

DK 不知道..... **(SKIP TO B3)** ..... 99. ☐

B2. a If YES, Why? (Specify reason) [neonarey]

_____

_____

B3. During the pregnancy with the child, did a doctor ever tell you that you had?

(**READ LIST**)

在怀着您的孩子时，医生有否曾经告诉您有关

（请读出）？

		YES 有	NO 没有	DK 不知道	During what month of pregnancy did the doctor first tell her this? 在怀孕期间的第几个月，医生第一次告诉他以下 (RF = 98, DK = 99) (拒绝回答 = 98, 不知道 = 99) **MONTH 月**
	(RF = 98, DK = 99) (拒绝回答 = 98, 不知道 = 99)				
1)	anemia or low blood count 贫血症或血球计数低 ..... [anemia]	1	0	99	☐ [anemth]
2)	high blood pressure that developed during pregnancy, but went away after the pregnancy was over 怀孕期间产生的高血压，但是怀孕后将不存在 ..... [hibp]	1	0	99	☐ [hibpmth]
3)	diabetes that developed during pregnancy, but went away after the pregnancy was over 怀孕期间产生的糖尿病，但是怀孕后将不存在 ..... [db]	1	0	99	☐ [dbmth]
4)	any other problem during the pregnancy 在怀孕期间的其它问题..... Specify 请说明: _____ [pregoth]	1	0	99	☐ [othmth]

[pregspf]

SECTION C: Smoking and Alcohol Intake During Pregnancy for this particular Child.

C 项：在怀着这孩子期间吃药、抽烟和喝酒

C 0. Is the person who answering the questionnaire the biological mother of the child?

受采访者是否是孩子的亲生母亲？

[biomo]

YES 是 ....1. ☐

NO 不是..... .....0. ☐

C 1. Did you take any medicines or traditional medicines during your pregnancy?

在怀孕的时候，您是否有吃药或传统中药？

[tradmed]

YES 有 ..... **(GO TO C 1a)**.....1. ☐

NO 没有.....**(SKIP TO C 2)**.....0. ☐

RF 拒绝回答 .....**(SKIP TO C 2)**.....98. ☐

DK 不知道 .....**(SKIP TO C 2)**.....99. ☐

C 1a. If YES, name all medicines

如果有，请列出药物的名称

[medspf]

SPECIFY 请说明:

(If traditional medicine, please list down the names of the medicines. If not, please write down herbs, e.g. Chinese herbs, Malay herbs.)

C 2. At any time during the pregnancy with the child, did you smoke?

在怀着您的孩子的任何时候，您有否抽烟？

[pregsmk]

YES 有 ..... **(GO TO C 2a)**.....1. ☐

NO 沒有.....**(SKIP TO C 3)**.....0. ☐

RF 拒绝回答 .....**(SKIP TO C 3)**.....98. ☐

DK 不知道 .....**(SKIP TO C 3)**.....99. ☐

C 2a. During which months of the pregnancy with the child did you smoke? [smkmth]

CODE ALL THAT APPLY.

在怀着您的孩子的哪一个月，您有抽烟？

记录所有选项。

MONTH 1 第一个月 .....1. ☐
MONTH 2 第二个月 .....2. ☐
MONTH 3 第三个月 .....3. ☐
MONTH 4 第四个月 .....4. ☐
MONTH 5 第五个月 .....5. ☐
MONTH 6 第六个月 .....6. ☐
MONTH 7 第七个月 .....7. ☐
MONTH 8 第八个月 .....8. ☐
MONTH 9 第九个月 .....9. ☐
RF 拒绝回答 .....98. ☐
DK 不知道 .....99. ☐

C 2b. On average, how many cigarettes per day did you smoke? [smkno]

您平均每天抽多少支香烟？

☐☐..... # CIGS PER DAY 每天多少支香
RF 拒绝回答 .....98. ☐
DK 不知道 .....99. ☐

C 3. At any time during the pregnancy with the child, did you drink alcohol? [pregalc]

在怀着您的孩子的任何时候，您有否喝酒？

YES 有 ..... **(GO TO C 3a)**.....1. ☐
NO 沒有..... **(SKIP TO D)**.....0. ☐
RF 拒绝回答 ..... **(SKIP TO D)**.....98. ☐
DK 不知道 ..... **(SKIP TO D)**.....99. ☐

C 3a. During which months of the pregnancy with the child did you drink

MONTH 1 第一个月 .....1. ☐

alcohol? [alcmth]

CODE ALL THAT APPLY.

在怀着您的孩子的哪一个月，您有喝酒？

记录所有选项。

MONTH 2 第二个月 .....2. ☐

MONTH 3 第三个月 .....3. ☐

MONTH 4 第四个月 .....4. ☐

MONTH 5 第五个月 .....5. ☐

MONTH 6 第六个月 .....6. ☐

MONTH 7 第七个月 .....7. ☐

MONTH 8 第八个月 .....8. ☐

MONTH 9 第九个月 .....9. ☐

RF 拒绝回答 .....98. ☐

DK 不知道 .....99. ☐

C 3b. During an average month during your pregnancy with the child, how many days in a week did you drink alcohol? [alcdays]

在怀着您孩子的期间，您平均每星期有多少天喝酒？

└┘ ..... # OF DAYS A WEEK 每星期多少天

OCCASIONAL DRINK / NO AVERAGE PATTERN

偶尔喝 / 不固定..... .....0. ☐

RF 拒绝回答 .....98. ☐

DK 不知道 .....99. ☐

C 3c. On average, how many drinks per day did you have? [alcdrink]

您平均每天喝多少杯酒？

└┘└┘. # DRINKS PER DAY 每天多少杯

RF 拒绝回答 .....98. ☐

DK 不知道 .....99. ☐

SECTION D: History of Health Conditions

D 项: 健康病历

			NAME1 姓名 1			
D1.	Has a doctor ever said that your child had (**READ LIST**)? 医生是否曾经说过您孩子有以下病症？（**读出以下各项**）？		YES 有	NO 没有	RF 拒绝回答	DK 不知道
	1. Asthma 哮喘……	[asthma]	1. ☐	2. ☐	98. ☐	99. ☐
	2. Chronic allergies or sinus trouble 长期的过敏症或额窦性问题……	[allergy]	1. ☐	2. ☐	98. ☐	99. ☐
	3. Mental retardation 智力迟钝 ……	[retard]	1. ☐	2. ☐	98. ☐	99. ☐
	4. Very high fever that caused convulsions or seizures 高烧所引起的抽搐或癫痫……	[fits]	1. ☐	2. ☐	98. ☐	99. ☐
	5. Coordination problem, motor delay, muscle weakness or paralysis 协调问题，运动神经迟缓，肌肉无力或瘫痪……	[paralyse]	1. ☐	2. ☐	98. ☐	99. ☐
	6. Any heart condition 任何心脏问题 ……	[heart]	1. ☐	2. ☐	98. ☐	99. ☐
	7. Speech or hearing problems 说话或听觉问题 ……	[speech]	1. ☐	2. ☐	98. ☐	99. ☐
	8. Attention or learning problems 注意力或学习问……	[learning]	1. ☐	2. ☐	98. ☐	99. ☐

9. Developmental delay 成长的延误 ……	[dvpdl]	1. ☐ 2. ☐ 98. ☐ 99. ☐
10. Diabetes 糖尿病……	[diab]	1. ☐ 2. ☐ 98. ☐ 99. ☐
11. Other problems 其它问题 ……	[othsprob]	1. ☐ 2. ☐ 98. ☐ 99. ☐

SPECIFY 详细说明:

[probspf1] [probspf2] [probspf3]

_____

_____

SECTION E: History of BREAST FEEDING: 哺乳的历史

E1. Was your child ever breastfed or fed breast milk? 您的孩子是否曾经被喂以母奶?

[bf]

0. ☐ No (If no, go to Section F)
没有（如果没有，请到 F 项）

99. ☐ Don't Know (If DK, go to Section F)
不知道（如果不知道，请到 F 项）

1. ☐ Yes (If yes, go to Question E2)
有（如果有，请回答问题 E2）

E2 How old was your child when (he/she) was first fed breast milk?

您的孩子第一次被喂以母奶时，他多大？

If < 1 month old ☐☐ days old

如果小过一个月 天大

[bfday]

If started breastfeeding 1 month or older ☐☐ months old

如果在一个月或以后开始喂以母奶 个月大

[bfmth]

E3 How long did you breastfeed this child? (For how long was your child breastfed or received breast milk?)

您喂母奶给这孩子多久了？（您的孩子喝多久的母奶？）

[bfdur]

1. ☐ Less than 1 week

少过一个星期

2. ☐ 1 to 4 weeks

一至四个星期

3. ☐ 1 to 3 months

一至三个月

4. ☐ 4 to 6 months

四至六个月

5. ☐ 6 to 12 months

六至十二个月

6. ☐ More than 12 months

多过十二个月

7. ☐ Still breastfeeding

还在喂以母奶

E4 Which type of breastfeeding best describes what you practiced at that time?

(Which type of breastfeeding best described what your child received at that time?)

哪一种喂以母奶的方式最适合形容您那个时候的做法？

（哪一种喂以母奶的方式最适合形容您孩子那个时候所得到的？）

[bfmtd1]

1. ☐ Exclusive breastfeeding (Only breast milk – may include medicines & vitamins)

纯粹喂以母奶 (只有母奶 – 可能包括药物或维他命)

2. ☐ Mostly breastfeeding (Breast milk and water, sweetened water, or juices– NO formula milk)

大部分喂以母奶 (母奶和水，糖水，或果汁 – 没有婴儿奶粉)

3. ☐ Partly breastfeeding (Breast milk AND formula milk or other complementary foods)

部分喂以母奶 (母奶和婴儿奶粉或其它补充的食物)

E5 How did you feed your child breast milk? (How did the mother feed the child breast milk?)

您怎样喂母奶给您孩子？（妈妈怎样喂母奶给孩子？）

[bfmtd2]

1. ☐ Directly from the breast (direct breastfeeding)

直接从乳房 (直接喂以母奶)

2. ☐ Expressed breast milk feedings without progression to direct breastfeeding

挤出母奶后才喂（不是直接喂以母奶）

3. ☐ Partial direct breastfeeding and partial expressed breast milk feedings

部分直接从乳房喂以母奶和部分挤出母奶后才喂

SECTION F: History of Ocular Conditions for the CHILD

F 项: 视觉病历

F1 During the past 12 months have you noticed that your child frequently squinting?

(i.e. eye turns in/out)

在过去的 12 个月里，您有否察觉您的孩子经常斜着眼看东西？(即当一只眼睛看向前面但另一只眼睛交叉或目光无目的地移动)

[squint]

YES 有..... 1. ☐
NO 没有..... 0. ☐
RF 拒绝回答..... 98. ☐
DK 不知道..... 99. ☐

IF CHILD IS < 24 MONTHS, SKIP TO F3.
如果孩子小于 24 个月，请跳到 F3

F2 During the past 12 months has your child had difficulty drawing or coloring, besides not staying in the lines?

在过去的 12 个月里，您的孩子有否绘画或填色方面的困难（不包括填色时越过线）？

[drawing]

NOT APPLICABLE 不适合……...2. ☐
YES 有..... 1. ☐
NO 没有..... 0. ☐
RF 拒绝回答..... 98. ☐
DK 不知道..... 99. ☐

F3 Does your child close one eye when (he/she) is in bright sun light?

当您的孩子在强烈阳光下时，（他/她）有否闭上一只眼睛？

[sunlight]

YES 有..... 1. ☐
NO 没有..... 0. ☐
RF 拒绝回答..... 98. ☐
DK 不知道..... 99. ☐

F4 Does your child close or cover one eye when (he/she) is concentrating on a task?

当他正专心做一件事情时，您的孩子有否闭上或遮盖一只眼睛？

[task]

YES 有..... 1. ☐
NO 没有..... 0. ☐
RF 拒绝回答..... 98. ☐
DK 不知道..... 99. ☐

F5 How often are your child's eyes checked?

[eyechk]

您孩子的眼睛是否经常接受检查？

This is the first time 这是第一次..... 1. ☐

6 months 6 个月 ..... 2. ☐

Once a year 一年一次 ..... 3. ☐

Once in 2 years 两年一次..... 4. ☐

Once in 3 years 三年一次..... 5. ☐

Once in 4 years 四年一次..... 6. ☐

Once in 5 years or more

五年一次或更多..... 7. ☐

RF 拒绝回答..... 98. ☐

DK 不知道..... 99. ☐

F6 Has a doctor told you that your child needs to wear glasses or contact lenses?

[needglas]

医生有否说过您的孩子需要佩戴眼镜或隐形眼镜？

YES 有..... **(GO TO F6 a)**..... 1. ☐

NO 没有..... **(SKIP F 7)** ..... 0. ☐

RF 拒绝回答..... **(SKIP F 7)** ..... 98. ☐

DK 不知道..... **(SKIP F 7)** ..... 99. ☐

F6a. And when?

[galsyrs]

什么时候？

└┴┘ ..... YEARS AGO 年前

RF 拒绝回答..... ..... 98. ☐

DK 不知道..... 99. ☐

F6b. Did your child get them?

[glasyes]

您的孩子是否有佩戴?

YES 有.....**(GO TO F6 d)**..... 1. ☐

NO 没有...**(GO TO F6c, THEN F 8)**0. ☐

RF 拒绝回答.....**(GO TO F 7)**..... 98. ☐

DK 不知道.....**(GO TO F 7)**..... 99. ☐

F6c. If not, why?

若没有，为什么？

[glasnot]

Adopt a wait and see approach because the prescription is too low

再观望一阵子，因为度数太低..... 1. ☐

Doctor / optometrist advice against glasses as they do not see the need for the child to wear glasses yet

医生 / 验光师反对佩戴眼睛，因为他们认为孩子还不需要配戴眼镜..... 2. ☐

Child does not like the idea of wearing glasses

孩子不喜欢配戴眼镜..... 3. ☐

The price of the spectacles was too expensive

眼镜的价格太昂贵 ..... 4. ☐

Cannot find any suitable frame

找不到适合的镜框 ..... 5. ☐

RF 拒绝回答..... 98. ☐

DK 不知道..... 99. ☐

F6d. When did your child first begin wearing glasses or contact lenses?

在什么时候，您的孩子第一次佩戴眼 镜或隐形眼镜？

[wearmth] [wearyrs]

MONTH 月 ☐☐

☐ RF 88 拒绝回答

☐ DK 99 不知道

YEAR 年 ☐☐☐☐

☐ RF 88 拒绝回答

☐ DK 99 不知道

IF RF, PROBE:
How old was your child when he/she first began wearing glasses or contact lenses?

如果拒绝回答, 调查:
您的孩子是在几岁时第一次佩戴眼镜或隐性眼镜？

[wearage]

OR 或

AGE 年龄 ☐☐

☐ RF 拒绝回答

☐ DK 不知道

F7 a) Does your child wear spectacles?

[wrspec]

您的孩子是否有佩戴眼镜?

YES 有..... **(GO TO F7 b)**..... 1. ☐

NO 没有..... **(SKIP TO F 8)**..... 0. ☐

RF 拒绝回答..... **(SKIP TO F 8)**..... 98. ☐

DK 不知道..... **(SKIP TO F 8)**..... 99. ☐

b) Does s/he need glasses primarily for:
(CHECK ONLY ONE)

[specneed]

他需要眼镜的主要目的是:

(只能选一项)

Viewing things clearly in the distance (eg, television or the blackboard

能在一定距离内看清事物（例如：电视或黑板）..... 1. ☐

Reading or other close work

阅读或看清其它较近的事物 ..... 2. ☐

Equally important for distance and close work

以上两项都一样重要..... 3. ☐

RF 拒绝回答..... 98. ☐

DK 不知道..... 99. ☐

c) Is the prescription fitted in the lenses the same as the prescription prescribed?

[sameprsc]

镜片的度数是否和验眼的度数是一样的?

YES 是..... **(SKIP TO F7 d)**..... 1. ☐

NO 不是..... **(Go TO F7 c) i)**..... 0. ☐

RF 拒绝回答.... **(SKIP TO F7 d)**.... 98. ☐

DK 不知道..... **(SKIP TO F7 d)**.... 99. ☐

i. If NO, is it generally lower?

[prscblow]

若不是，是不是比一般的低呢?

YES 是..... **(SKIP TO F7 c) ii)**.... 1. ☐

NO 不是..... **(SKIP TO F7 d)**..... 0. ☐

RF 拒绝回答.... **(SKIP TO F7 d)**.... 98. ☐

DK 不知道..... **(SKIP TO F7 d)**.... 99. ☐

ii. If lower, who requested it?

如较低，是谁要求的呢？

[prscreq]

Prescriber 给与处方的人 ..... 1. ☐

Parents 家长 .... 0. ☐

RF 拒绝回答..... ..... 98. ☐

DK 不知道..... 99. ☐

d) On an average day, how many hours per day does your child wear glasses?

平均一天里，你的孩子会戴眼镜几个小时？

[glashrs]

☐☐ .....Hours / Day 小时/天

RF 拒绝回答..... ..... 98. ☐

DK 不知道..... 99. ☐

e) If your child wears spectacles, does your child wear glasses when playing sports?

如果您的孩子有佩戴眼镜，在运动时，他有否戴着眼镜 ？

[glasplay]

YES 有..... 1. ☐

NO 没有 .... 0. ☐

RF 拒绝回答..... ..... 98. ☐

DK 不知道..... ..... 90. ☐

Amblyopia is poor vision in an eye that cannot be corrected with glasses or contact lenses and the eye looks normal.

弱视是一种视力的缺陷，不能即由眼镜或隐性眼镜而矫正，而眼睛也看似正常。

F8 1) Has a doctor ever told you that your child had amblyopia?

医生有否说过您的孩子有弱视的问题？

[ambly]

YES 有.....**(GO TO F8 (2))**..... 1. ☐

NO 没有 ..... **(SKIP TO F 9)**..... 0. ☐

RF 拒绝回答.... **(SKIP TO F 9)**..... 98. ☐

DK 不知道..... **(SKIP TO F 9)**..... 99. ☐

2) When was your child first diagnosed as having amblyobia?

[amblymth]
[amblyyrs]

☐☐ MONTH 月 ☐☐☐☐ YEAR 年

在什么时候，您的孩子第一次被诊断出患有弱视？

IF RF, PROBE:

How old was your child when he/she was first diagnosed? [amblyage]

如果拒绝回答, 调查:

您的孩子是在几岁时第一次被诊断出患有弱视？

☐ RF 拒绝回答 ☐ RF 拒绝回答

☐ DK 不知道 ☐ DK 不知道

OR 或 AGE 年龄 ☐☐

☐ RF 拒绝回答

☐ DK 不知道

3) Was that in your child's right eye, left eye, or both eyes? [amblyeye]

您孩子的右眼，左眼或双眼患有弱视？

RIGHT EYE 右眼 ..... 1. ☐

LEFT EYE 左眼..... 2. ☐

BOTH EYES 双眼 ..... 3. ☐

RF 拒绝回答..... ..... 98. ☐

DK 不知道..... ..... 99. ☐

4) Has your child ever been treated for amblyopia? [amblytx]

您的孩子有否接受过弱视的治疗？

YES 有.....**(GO TO F8 (5))**..... 1. ☐

NO 没有 ..... **(SKIP TO F 9)**..... 0. ☐

RF 拒绝回答..... **(SKIP TO F 9)**..... 98. ☐

DK 不知道..... **(SKIP TO F 9)**..... 99. ☐

5) What treatment or treatments did your child receive?
READ ITEMS.

以下哪些是您的孩子接受过的治疗?

读出以下各项

		YES 有	NO 没有	RF 拒绝回答	DK 不知道
a) Glasses or contact lenses 眼镜或隐形眼镜	[txglass]	1. ☐	0. ☐	98. ☐	99. ☐
b) Patching (保護病傷眼睛用的)眼罩 ...	[txpatch]	1. ☐	0. ☐	98. ☐	99. ☐
c) Eye drops 眼药水	[txdrops]	1. ☐	0. ☐	98. ☐	99. ☐

d) Vision therapy 视力疗法..... [txvision] 1. ☐ 0. ☐ 98. ☐ 99. ☐

e) Other 其它..... [txoths] 1. ☐ 0. ☐ 98. ☐ 99. ☐

SPECIFY 详细说明:

[txspec] _____

6) How long did your child receive treatments? [txdur]

您的孩子接受治疗有几年了？

└┴┘ ..... YEARS 年

RF 拒绝回答..... ..... 98. ☐

DK 不知道..... 99. ☐

7) Is the child still undergoing the treatment or has it been stopped? [txstatus]

您的孩子还继续接受治疗吗？还是已经停止了？

STILL UNDERGOING 还继续接受治疗

.....**(SKIP TO F 9)**..... 1. ☐

STOPPED 已经停止了

.....**(GO TO F 8 (8))**..... 2. ☐

RF 拒绝回答..... ..... 98. ☐

DK 不知道..... ..... 99. ☐

8) If stopped, why was the treatment stopped? [txstop]

若已经停止了，是什么原因呢？

_____

_____

_____

RF 拒绝回答..... ..... 98. ☐

DK 不知道..... ..... 99. ☐

Strabismus – Eyes that are not properly lined up. This happens when one eye looks straight

ahead and the other eye crosses in or wanders out.

斜视 – 两只眼睛不能完全地列好，即当一只眼睛看向前面但另一只眼睛交叉或目光无目的地移动。

F9 1) Does your child have Strabismus? [strab]

您的孩子有否患上斜视？

YES 有.....**(GO TO F9 (2))**..... 1. ☐

NO 没有.....**(SKIP TO G 1)**..... 0. ☐

RF 拒绝回答.....**(SKIP TO G 1)**.... 98. ☐

DK 不知道.....**(SKIP TO G 1)**.... 99. ☐

2) When was your child first diagnosed as having strabismus? [strabmth] [strabyrs]

在什么时候，您的孩子第一次被诊断出患有斜视？

MONTH 月 ☐☐ YEAR 年 ☐☐☐☐

☐ RF 拒绝回答 ☐ RF 拒绝回答

☐ DK 不知道 ☐ DK 不知道

IF RF, PROBE:

How old was your child when he/she was first diagnosed? [strabage]

如果拒绝回答, 调查:

您的孩子是在几岁时第一次被诊断出患有斜视？

OR 或 AGE 年龄 ☐☐

☐ RF 拒绝回答

☐ DK 不知道

3) Was that in your child's right eye, left eye, or both eyes? [strabeye]

您孩子的右眼，左眼或双眼患有斜视？

RIGHT EYE 右眼 ..... 1. ☐

LEFT EYE 左眼..... 2. ☐

BOTH EYES 双眼 ..... 3. ☐

RF 拒绝回答..... ..... 98. ☐

DK 不知道..... ..... 99. ☐

4) Has your child ever been treated for Strabismus?

[strabtx]

您的孩子有否接受过斜视的治疗？

YES 有..... **(GO TO F9 (5))** ..... 1. ☐

NO 没有.....**(SKIP TO G 1)**..... 0. ☐

RF 拒绝回答.....**(SKIP TO G 1)**.... 98. ☐

DK 不知道.....**(SKIP TO G 1)**.... 99. ☐

5) What kind of treatment did your child receive?

[stratxtyp]

您的孩子接受过什么治疗？

_____

_____

_____

RF 拒绝回答..... ..... 98. ☐

DK 不知道..... 99. ☐

Section G: Outdoor and indoor and pre-school activities

G 项: 室外、室内及就学前的活动

For Question G 1a, 1b and 1c, the total time spend should be *24* hours.

问题 G 1a，1b 和 1c，其时间的和应为 *24* 小时。

G1 a) On a normal 24 hour day, how many hours per day does your child spend sleeping?

[sleep]

一天 24 小时里，您的孩子睡多少个小时？

每天 ☐☐ 小时 hrs per day

RF 拒绝回答..... .....98. ☐

DK 不知道..... .....99. ☐

b) On a normal 24 hour day, How many hours per day does your child spend indoors (example at home, friend's house, shopping) ?

[indoor]

一天 24 小时里，您的孩子花在室内的

每天 ☐☐ 小时 hrs per day

RF 拒绝回答..... .....98. ☐

DK 不知道..... .....99. ☐

时间有几个小时？（例如在家、朋友的家、逛街）

c) On a normal 24 hour day, how many hours per day does your child spend outdoors (i.e. not in an enclosed space)? [outdoor]

一天 24 小时里，您的孩子花在户外的时间有几个小时?（室外:不在密闭的空间）

每天 ⌊_⌋⌊_⌋ 小时 hrs per day

RF 拒绝回答..... .....98. ☐

DK 不知道..... .....99. ☐

Please specify the sports (e.g. swimming, Tennis) your child do and the **number of hours per week** during the school term that your child spend doing the activity.

请指名那些运动（例如游泳，网球）您孩子**（姓名）**参与的及在学校里每星期有多少小时参与这些运动。

G 2. OUTDOOR SPORTS DURING THE 7 DAYS OF THE WEEK {for your child (NAME)}

您孩子（姓名）在一星期七天内的室外运动

	Name of the outdoor sports **室外运动的名称**	Number of hours per week spent in this activity 每星期多少小时参与这项运动
1	[sports1]	每星期 ⌊_⌋⌊_⌋ 小时 hrs per week [spthrs1]
2	[sports2]	每星期 ⌊_⌋⌊_⌋ 小时 hrs per week [spthrs2]
3	[sports3]	每星期 ⌊_⌋⌊_⌋ 小时 hrs per week [spthrs3]
4	[sports4]	每星期 ⌊_⌋⌊_⌋ 小时 hrs per week [spthrs4]

G 3. **During the school year, how many hours per day (outside of regular school hours) would you estimate your child:**

在上学学年里，您的孩子每天大约花多少小时（不包括正规的上课时间）：

(PLEASE tick ✓ "Not Applicable" if your child does not perform this activity)

(请画 ✓ "不适当" 如果您的孩子没有参与此项活动)

	On the weekdays (Mon.-Fri.) 周日（星期一至五)	On the weekend (Sat. & Sun.) 周末（星期六及日)	Not applicable 不适当	Refused 拒绝	DK 不知道
a) Reading and writing (school work & read for pleasure) 读书和写字(课业及娱乐)	⌊__⌋ hours/day 小时/天 **[wdrw]**	⌊__⌋ hours/day 小时/天 **[wkrw]**	0	98	99
b) Colors, or draws for fun (pleasure) 填色，画画（娱乐）	⌊__⌋ hours/day 小时/天 **[wdcolor]**	⌊__⌋ hours/day 小时/天 **[wecolor]**	0	98	99
c) Watches television 看电视	⌊__⌋ hours/day 小时/天 **[wdtv]**	⌊__⌋ hours/day 小时/天 **[wetv]**	0	98	99
d) Playing television games (e.g. play station) 玩电子游戏 (例如：play station)	⌊__⌋ hours/day 小时/天 **[wdtvgame]**	⌊__⌋ hours/day 小时/天 **[wetvgame]**	0	98	99
e) Uses a computer / plays computers 用电脑 / 玩电脑	⌊__⌋ hours/day 小时/天 **[wdcomp]**	⌊__⌋ hours/day 小时/天 **[wecomp]**	0	98	99

f) Plays hand held video games (e.g. gameboy, handphone games). 玩手提式电动游戏（例如：gameboy, 电话游戏)	⌊_⌋ hours/day 小时/天 [wdvideo]	⌊_⌋ hours/day 小时/天 [wevideo]	0	98	99
g) Other near work activities, please describe below: (e.g. cutting paper, playing with toys) 其它活动，请说明（如剪纸，玩具）： [nwtype] _____ _____	⌊_⌋ hours/day 小时/天 [wdoths]	⌊_⌋ hours/day 小时/天 [weoths]	0	98	99
Time Spent outside 室外活动的时间：					
h) Playing out of doors (In a backyard, walk, bike riding) 室外游戏 (在后院, 散步, 骑脚车)	⌊_⌋ hours/day 小时/天 [wdplay]	⌊_⌋ hours/day 小时/天 [weplay]	0	98	99
i) Out door leisure activities (Family BBQs, Park, Picnic, Beach) 室外闲暇活动 (家庭烤肉, 公园, 野餐, 海边)	⌊_⌋ hours/day 小时/天 [wdleis]	⌊_⌋ hours/day 小时/天 [weleis]	0	98	99

G 4. Is there a park or garden near to your home where your child could play outdoors?

在您家附近是否有公园或花园（您孩子可到那里去玩）？

[garden]

YES 有 ..... **(GO TO G4 a)** ..... 1. ☐

NO 没有..... **(SKIP TO G 5)** ..... 0. ☐

RF 拒绝回答 ..... **(SKIP TO G 5)** ..... 98. ☐

DK 不知道..... **(SKIP TO G 5)** ..... 99. ☐

G 4a. If YES, does your child play in the nearby

YES 有 ..... 1. ☐

NO 没有..... 0. ☐

park or garden at least once a week?

若是，您的孩子是否至少一个星期到该公园或花园玩一次？

[freqgdn]

RF 拒绝回答 ..... 98. ☐

DK 不知道..... 99. ☐

G 5. Does your child read words by himself or herself?

(A "word" can be a letter (e.g. A, B, C) or a word (e.g. Apple))

您的孩子有否自行阅读单字？

（（一个单字可以是一个字母（如：A，B，C）或一个字（如：Apple））

[wordslf]

YES 有 ..... 1. ☐

NO 没有..... 0. ☐

RF 拒绝回答 ..... 98. ☐

DK 不知道..... 99. ☐

G 6. Does your child read picture books by himself or herself

您的孩子有否自行阅读图画书？

[pictslf]

YES 有 ..... 1. ☐

NO 没有..... 0. ☐

RF 拒绝回答 ..... 98. ☐

DK 不知道..... 99. ☐

G 7. How often does your child read for fun (outside of school)?

(CHECK ONLY ONE BOX)

您的孩子是否经常自行阅读（在学校以外）？

(只能选一项)

[funread]

Never 从来没有.....**(SKIP TO G 12)**..... 1. ☐

Rarely 很少 ..... 2. ☐

Sometimes 有时 ..... 3. ☐

Often 经常 ..... 4. ☐

Child doesn't read yet 还未开始阅读

.....**(SKIP TO G 12)**..... 5. ☐

RF 拒绝回答 .....**(SKIP TO G 12)**..... 98. ☐

DK 不知道.....**(SKIP TO G 12)**..... 99. ☐

G 8. How long on average does your child read before taking a break?

您孩子平均会阅读多久才休息一会儿?

[readhrs]

0 – 10 minutes 分钟	1. ☐
11 – 20 minutes 分钟	2. ☐
21 – 30 minutes 分钟	3. ☐
31 – 40 minutes 分钟	4. ☐
41 – 50 minutes 分钟	5. ☐
51 – 60 minutes 分钟	6. ☐
> 60 minutes 超过 60 分钟	7. ☐
RF 拒绝回答	98. ☐
DK 不知道	99. ☐

G 9. How frequently does your child read with the book close to his or her face?

(Demonstrate reading at ~ 33 cm)

您孩子有否经常在阅读时把书靠近他的脸?

(示范读书在~ 33 cm)

[readclse]

NEVER 从未	0. ☐
SELDOM 很少	1. ☐
OFTEN 经常	2. ☐
NA 不适当	3. ☐
RF 拒绝回答	98. ☐
DK 不知道	99. ☐

	Has not started this activity 还没开始这项活动	RF 拒绝回答	DK 不知道
G 10. At what age did your child first start reading books by himself / herself on a regular basis 您的孩子多少岁开始自行地及习惯性地阅读 ☐☐ years old 岁 [agebook]	97. ☐	98. ☐	99. ☐
G 11. Number of books read per week {**Read by the Child**} ☐☐ books per	97. ☐	98. ☐	99. ☐

	一星期读多少本书{**由孩子读**}	week 本/每星期 **[nobkwk]**			
G 12.	Number of hours of academic tuition classes (e.g. school related subjects such as English. Math, Chinese) outside school hours 一星期多少小时的课外补习课（例如：英文科目数学、中文）	☐☐ hours per week 小时/每星期 **[hrtuitn]**	97. ☐	98. ☐	99. ☐
G 13.	At what age did your child start attending a pre-school centre (includes kindergarten, childcare and montessori centers) 您的孩子多少岁开始进入就学前的学习中心（例如：幼稚园、育幼院和蒙特梭利中心）	☐☐ years old 岁 **[preschyr]**	97. ☐ **SKIP TO H**	98. ☐	99. ☐

G 14. What type of preschool is your child attending?

现在您的孩子进入那一种就学前的学习中心?

[presch]

[prespf]

KINDERGARDEN 幼儿园 ..... 1. ☐

CHILDCARE 育幼院 ..... 2. ☐

NURSERY 托儿所 ..... 3. ☐

OTHERS 其它 ..... **(SPECIFY)** ..... 4. ☐

SPECIFY 请说明: _____

NONE 没有 ..... **(SKIP TO H)** ..... 5. ☐

RF 拒绝回答 ..... **(SKIP TO H)** ..... 98. ☐

DK 不知道 ..... **(SKIP TO H)** ..... 99. ☐

G 15. How many hours per day does your child spend in pre-school?

您孩子每天多少个小时在就学前的学习中

☐☐ hours per day 小时/每天

RF 拒绝回答 ..... 98. ☐

DK 不知道 ..... 99. ☐

心?

[preschhr]

SECTION H

H 项

		NAME1 姓名 1			
				I.	II.
H	Has a doctor ever told you that your child ever had (**READ LIST**)? 医生是否曾经说过您的孩子有以下病症？（读出以下各项） **RF=98, DK=99.** **拒绝回答=98, 不知道=99.**	YES (ASK I & II) 是 (发问 I & II)	NO 不是	What treatment was received? **IF NONE, SKIP TO NEXT CONDITION.** 接受什么治疗？ **如果没有，跳到下一项.**	When was this? 什么时候？
[cat]	1) Cataracts 白內障.....	1	0	_____ [cattx]	_____ [catdur]
[glau]	2) Glaucoma 青光眼，綠內障.....	1	0	_____ [glautx]	_____ [glaudur]
[ret]	3) Retinopathy of prematurity ..... 早产儿视网膜病	1	0	_____ [rettx]	_____ [retdur]
[tumor]	4) Eye tumor or retinoblastoma ..... 眼睛肿瘤或視網膜神經膠質瘤（即眼癌，遗传性）	1	0	_____ [tumortx]	_____ [tumordur]
[opticnr]	5) Optic nerve hypoplasia..... 视力神经发育不全	1	0	_____ [optictx]	_____ [opticdur]
[ductob]	6) Nasolacrimal duct obstruction ..... 属于鼻与泪器的輸送管梗塞	1	0	_____ [ducttx]	_____ [ductdur]
	7) Cortical visual impairment	1	0	_____	_____

[cortvi]	皮层视力损伤	1	0	[corttx]	[cortdur]
[eyeoths]	8) Others. Please specify: 其它（请详细说明） _____	1	0	_____ [eyeothtx]	_____ [eyeotdur]

SECTION I (I 项):

Developmental Delay: 成长的延误

[dvpln]

1) Do you have any concerns about your child's learning, development, and behavior?

您是否担心您孩子的学习，成长及行为?

YES 是.....1. ☐

A LITTLE 一点.....2. ☐

NO 不是 ..... **(SKIP TO I 3)**.....0. ☐

RF 拒绝回答..... **(SKIP TO I 3)**.....98. ☐

DK 不知道 ..... **(SKIP TO I 3)**.....99. ☐

[lrncrn]

2) What are your concerns? **CODE ALL THAT APPLY.**

您担心的是什么?

记录所有选项。

SEEMS BEHIND

似乎落后 .....1. ☐

CAN'T DO WHAT OTHER KIDS CAN

不可以做其他孩子做的事.....2. ☐

SLOW AND BEHIND OTHER KIDS

比其他孩子慢及落后.....3. ☐

IMMATURE

不成熟.....4. ☐

LEARNS SLOWLY

学习能力慢.....5. ☐

LATE TO LEARN TO DO THINGS

迟开始学习 .....6. ☐

LEARNS BUT TAKES A LONG TIME

需要较长的时间学习 .....7. ☐

PROBLEMS LEARNING EVERYTHING

学习任何事情都有问题.....8. ☐

OTHER (SPECIFY)

其它 (请说明).....9. ☐

SPECIFY: _____

请说明

RF 拒绝回答.....98. ☐

DK 不知道 .....99. ☐

[lrncrn1]

SPEECH : 说话及发音

[dvpsph]

3) Do you have any concerns about how your child talks and makes speech sounds?

您是否担心您孩子的说话及发音？

YES 是.....1. ☐

A LITTLE 一点.....2. ☐

NO 不是 ..... **(SKIP TO I 5)**.....0. ☐

RF 拒绝回答..... **(SKIP TO I 5)**.....98. ☐

DK 不知道 ..... **(SKIP TO I 5)**.....99. ☐

[sphcrn]

4) What are your concerns? **CODE ALL THAT APPLY.**

您担心的是什么？

记录所有选项。

NOT TALKING LIKE HE/SHE SHOULD

不能照他的方式说话.....1. ☐

USES SHORT SENTENCES

用缩短的句子表达.....2. ☐

CAN'T ALWAYS SAY WHAT HE/SHE MEANS

不能表达他要表达的意思.....3. ☐

DOESN'T ALWAYS MAKE SENSE

不能让人明白 .....4. ☐

CAN'T TALK CLEARLY

不能清楚地说话 .....5. ☐

NOBODY UNDERSTANDS WHAT HE/SHE IS SAYING EXCEPT FAMILY MEMBERS

没有人了解他说什么，除了他的家人.....6. ☐

OTHER (SPECIFY)

其它 (请说明).....7. ☐

SPECIFY: _____

请说明

RF 拒绝回答.....98. ☐

DK 不知道 .....99. ☐

[sphcrn1]

COMPREHENSION : 领悟能力

[dvpund]

5) Do you have any concerns about how your child understands what you say?

您是否担心您孩子能否了解您说的话?

YES 是.....1. ☐

A LITTLE 一点.....2. ☐

NO 不是 ..... **(SKIP TO I 7)**.....0. ☐

RF 拒绝回答..... **(SKIP TO I 7)**.....98. ☐

DK 不知道 ..... **(SKIP TO I 7)**.....99. ☐

[undcrn]

6) What are your concerns? **CODE ALL THAT APPLY.**

您担心的是什么?

记录所有选项。

DOESN'T UNDERSTAND WHAT YOU SAY

不明白你说什么 .....1. ☐

DOESN'T LISTEN WELL

不能听好 .....2. ☐

OTHER (SPECIFY)

其它 (请说明).....3. ☐

SPECIFY: _____

[undcrn1]

请说明

RF 拒绝回答 ..... 98. ☐

DK 不知道 ..... 99. ☐

FINE MOTOR SKILLS: 精细的运动技巧

[dvphnd]

7) Do you have any concerns about how your child uses his or her hands and fingers to do things?

您是否担心您孩子怎样使用他的手和手指做事？

YES 是 ..... 1. ☐

A LITTLE 一点 ..... 2. ☐

NO 不是 ..... **(SKIP TO I 9)** ..... 0. ☐

RF 拒绝回答 ..... **(SKIP TO I 9)** ..... 98. ☐

DK 不知道 ..... **(SKIP TO I 9)** ..... 99. ☐

[hndcrn]

8) What are your concerns? **CODE ALL THAT APPLY.**

您担心的是什么？

记录所有选项。

CAN'T STAY IN LINES WHEN COLORS

填色时超越线 ..... 1. ☐

CAN'T WRITE NAME

不能写自己的名字 ..... 2. ☐

CAN'T DRAW SHAPES

不能画形状 ..... 3. ☐

CAN'T HOLD A PENCIL RIGHT

不能正确地握笔

..... 4. ☐

CAN'T GET FOOD TO MOUTH/MESSY EATER

不能把食物送进嘴里 ..... 5. ☐

OTHER (SPECIFY)

其它 (请说明) ..... 6. ☐

SPECIFY: _____

请说明

RF 拒绝回答 ..... 98. ☐

DK 不知道 ..... 99. ☐

[hndcrn1]

GROSS MOTOR SKILLS : 整体的运动技巧

[dvparm]

9) Do you have any concerns about how your child uses his or her arms and legs?

您是否担心您孩子怎样使用他的手臂和脚？

YES 是 ..... 1. ☐

A LITTLE 一点 ..... 2. ☐

NO 不是 ..... **(SKIP TO I 11)** ..... 0. ☐

RF 拒绝回答 ..... **(SKIP TO I 11)** ..... 98. ☐

DK 不知道 ..... **(SKIP TO I 11)** ..... 99. ☐

[armcrn]

10) What are your concerns? **CODE ALL THAT APPLY.**

您担心的是什么？

记录所有选项。

CLUMSY 笨拙 ..... 1. ☐

WALKS FUNNY 走路滑稽 ..... 2. ☐

CAN'T RIDE A BIKE YET 还不能骑脚踏车 ... 3. ☐

FALLS A LOT 时常跌倒 ..... 4. ☐

LIMPS 跛行 ..... 5. ☐

POOR BALANCE 平衡能力差 ..... 6. ☐

OTHER (SPECIFY) 其它 (请说明) ..... 7. ☐

SPECIFY: _____

请说明

[armcrn1]

RF 拒绝回答 ..... 98. ☐

DK 不知道 ..... 99. ☐

BEHAVIOUR : 行为

[dvpbhv]

11) Do you have any concerns about how your child behaves?

您是否担心您孩子的行为？

YES 是 ..... 1. ☐

A LITTLE 一点 ..... 2. ☐

NO 不是 ..... **(SKIP TO I 13)** ..... 0. ☐

RF 拒绝回答 ..... **(SKIP TO I 13)** ..... 98. ☐

DK 不知道 ..... **(SKIP TO I 13)** ..... 99. ☐

STUBBORN 固执 ..... 1. ☐

[bhvcrn] 12) What are your concerns?
CODE ALL THAT APPLY.

您担心的是什么？

记录所有选项。

OVER-ACTIVE 过动.....2. ☐

SHORT ATTENTION SPAN 三分钟热度.....3. ☐

SPOILED 被宠坏.....4. ☐

AGGRAVATING 可恼的、讨厌的.....5. ☐

THROWS FITS 痉攣.....6. ☐

ONLY DOES WHAT HE/SHE WANTS

只做他想做的 .....7. ☐

OTHER (SPECIFY) 其它 (请说明).....8. ☐

SPECIFY: _____

请说明

RF 拒绝回答.....98. ☐

[bhvcrn1] DK 不知道 .....99. ☐

SOCIAL FUNCTIONING：社交技巧

[dvpgot] 13) Do you have any concerns about how your child gets along with others?

您是否担心您孩子怎么与人相处？

YES 是.....1. ☐

A LITTLE 一点.....2. ☐

NO 不是 ..... **(SKIP TO I 15)**.....0. ☐

RF 拒绝回答..... **(SKIP TO I 15)**.....98. ☐

DK 不知道 ..... **(SKIP TO I 15)**.....99. ☐

[gotcrn] 14) What are your concerns?
CODE ALL THAT APPLY.

您担心的是什么？

记录所有选项。

WANTS TO BE LEFT ALONE 只想一人独处1. ☐

MOOD SWINGS, CLINGY

心情摇摆不定，缠着人.....2. ☐

WHINY 爱抱怨 .....3. ☐

BOTHERED BY CHANGES 由于改变而烦恼4. ☐

ANGRY, DISINTERESTED IN USUAL THINGS

生气，对平常事没有兴趣 ..... 5. ☐

EASILY LEAD 容易被人带坏 ..... 6. ☐

ACTS MEAN 行为小气 ..... 7. ☐

EASILY FRUSTRATED 容易发怒 ..... 8. ☐

BOSSY 爱指挥他人的 ..... 9. ☐

SHY 害羞 ..... 10. ☐

CLASS CLOWN 诙谐的人 ..... 11. ☐

ANGRY 生气 ..... 12. ☐

MEAN 吝啬 ..... 13. ☐

HATES ME 讨厌我 ..... 14. ☐

OTHER (SPECIFY) 其它 (请说明)..... 15. ☐

SPECIFY: _____

请说明

RF 拒绝回答 ..... 98. ☐

DK 不知道 ..... 99. ☐

[gotcrn1]

LEARNING：学习做事

[dvpthgs]

15) Do you have any concerns about how your child is learning to do things for (himself/herself)?

否担心您孩子怎么为他们自己学习做事？

YES 是 ..... 1. ☐

A LITTLE 一点 ..... 2. ☐

NO 不是 ..... **(SKIP TO I 17)** ..... 0. ☐

RF 拒绝回答 ..... **(SKIP TO I 17)** ..... 98. ☐

DK 不知道 ..... **(SKIP TO I 17)** ..... 99. ☐

[thgscrn] 16) What are your concerns?
CODE ALL THAT APPLY.

您担心的是什么？

记录所有选项。

WON'T DO THINGS FOR HIM/HERSELF

不会为他们自己做事 ..... 1. ☐

WON'T TELL ME WHEN HE/SHE IS WET

当他撒尿他不会告诉我 ..... 2. ☐

NOT TOILET TRAINED YET

还没训练他上厕所 ..... 3. ☐

STILL WANTS A BOTTLE

还是要奶瓶 ..... 4. ☐

CAN'T GET DRESSED BY HIM/HERSELF

不会自己穿衣 ..... 5. ☐

OTHER (SPECIFY)

其它 (请说明) ..... 15. ☐

SPECIFY: _____

请说明

[thgscrn1]

RF 拒绝回答 ..... 98. ☐

DK 不知道 ..... 99. ☐

PRESCHOOL SKILLS : 学前技能

[dvpprsch] 17) Do you have any concerns about how your child is learning preschool or school skills?

您是否担心您孩子怎么在学习中心学习？

YES 是 ..... 1. ☐

A LITTLE 一点 ..... 2. ☐

NOT APPLICABLE 不适合 ..... 3. ☐

NO 不是 ..... **(SKIP TO I 19)** ..... 0. ☐

RF 拒绝回答 ..... **(SKIP TO I 19)** ..... 98. ☐

DK 不知道 ..... **(SKIP TO I 19)** ..... 99. ☐

[prschcrn] 18) What are your concerns?
CODE ALL THAT APPLY.

CAN'T WRITE HIS/HER NAME

您担心的是什么？

记录所有选项。

[prschcn1]

不会写他的名字 ..... 1. ☐

DOESN'T KNOW COLORS OR NUMBERS

不会辨认颜色和数字 ..... 2. ☐

JUST NOT LEARNING TO READ

不愿学习阅读 ..... 3. ☐

CAN'T REMEMBER LETTER SOUNDS

不记得字母的发音 ..... 4. ☐

KNOWS SPELLING WORDS ONE DAY BUT NOT THE NEXT

今天知道字的发音，第二天就忘了 ..... 5. ☐

OTHER (SPECIFY)

其它 (请说明) ..... 15. ☐

SPECIFY: _____

请说明

RF 拒绝回答 ..... 98. ☐

DK 不知道 ..... 99. ☐

Other Concerns : 其它担心的事情

[dvpoth]

19) Do you have any other concerns about your child?

您是否在其它事情上也担心您的孩子？

YES 是 ..... 1. ☐

A LITTLE 一点 ..... 2. ☐

NO 不是 ..... **(SKIP TO SECTION J)** ..... 0. ☐

RF 拒绝回答 .. **(SKIP TO SECTION J)** ..... 98. ☐

DK 不知道 ..... **(SKIP TO SECTION J)** ..... 99. ☐

[othcrn]

20) What are your concerns? **CODE ALL THAT APPLY.**

EAR INFECTIONS 耳朵传染 ..... 1. ☐

您担心的是什么？

记录所有选项。

ASTHMA 哮喘 ..... 2. ☐

SMALL FOR AGE

身材比实际年龄矮小 ..... 3. ☐

SICK A LOT 经常生病 ..... 4. ☐

I DON'T THINK HE/SHE HEARS WELL

我不认为他听觉好 ..... 5. ☐

HE/SHE GETS UP TOO CLOSE TO THE TV AND I WORRY ABOUT HIS/HER SIGHT

太靠近电视机，我担心他的视力 ..... 6. ☐

OTHER (SPECIFY) 其它 (请说明) ..... 15. ☐

SPECIFY: _____

请说明

RF 拒绝回答 ..... 98. ☐

[othcrn1]

DK 不知道 ..... 99. ☐

SECTION J: Please collect the following data from Health Booklet

J 项：请从健康手册集合以下资料

J 1 How much did your child weigh at birth? [bthwt]

在出世时，您的孩子的重量是多少？

WEIGHT AT BIRTH ..... ☐☐☐☐ gm

出世时的重量

RF 拒绝回答 ..... 98. ☐

DK 不知道 ..... 99. ☐

J 2 What was your child's gestational age at birth? [gesage]

在出世前，您的孩子在母体内待了多少个星期？

☐☐+☐ ..... # WEEKS 星期

RF 拒绝回答 ..... 98. ☐

DK 不知道 ..... 99. ☐

J What was your child's length at birth?

☐☐.☐ RF 拒绝回答....98. ☐

3	在出世时，您的孩子的身长是多少？	[blength]	Cm 公分 DK 不知道…….99. ☐
J 4	What was your child's Head circumference at birth? 在出世时，您的孩子的头的周长是多少？	[hdcf]	☐☐.☐ Cm 公分 RF 拒绝回答….98. ☐ DK 不知道…….99. ☐

INTERVIEWER ID: ☐☐☐ **END TIME** ☐☐:☐☐ AM/PM

STUDY ID: □□□□ _ □ _ □□

[sno] (Home ID) (Fam ID) (Child ID)

INTERVIEWER ID: □□□

[invest1]

Date of Interview: _____

[date1] (DD – MM – YYYY)

A Study on Strabismus, Amblyopia and Refractive Error in Singapore Preschoolers

(STARS Study)

新加坡学龄前孩童于斜视，弱视和折射误差方面的研究

Family History
家庭的来历

(1 Interview / Household)
(1 采访 / 家庭)

CHILD Sticky Label Here

Remarks 备注:

_____

_____

_____

A: Income and Educational Status

A 项: 收入和学历

A.1 What is child's father dialect group?
孩子爸爸的籍贯?

[fadial]

HOKKIEN 福建人 .....1. ☐

TEOCHEW 潮州人 .....2. ☐

CANTONESE 广东人 .....3. ☐

HAKKA 客家人.....4. ☐

HAINANESE 海南人.....5. ☐

HOKCHEW 福州人.....6. ☐

OTHER **(SPECIFY)** 其他（请说明）

.....7. ☐

Specify: _____

请说明

RF 88. ☐ DK 99. ☐

RF 拒绝回答.....98. ☐

[fadialsp]

DK 不知道.....99. ☐

A.2 What is child's mother dialect group?
孩子妈妈的籍贯？

[modial]

HOKKIEN 福建人 .....1. ☐

TEOCHEW 潮州人 .....2. ☐

CANTONESE 广东人 .....3. ☐

HAKKA 客家人.....4. ☐

HAINANESE 海南人.....5. ☐

HOKCHEW 福州人.....6. ☐

OTHER **(SPECIFY)** 其他（请说明）

.....7. ☐

Specify: _____

请说明

RF 88. ☐ DK 99. ☐

RF 拒绝回答.....98. ☐

[modialsp]

DK 不知道.....99. ☐

A.3 What is your total combined monthly household income?

(Singapore dollars)

您每月的家庭总收入是多少？

（以新元计算）

[income]

Less than S$1,000 少过 S$1,000.....1. ☐

S$1,000 – S$2,999 .....2. ☐

S$3,000 – S$4,999 .....3. ☐

S$5,000 and above

S$5,000 及以上.....4. ☐

RF 拒绝回答.....98. ☐

DK 不知道.....99. ☐

A.4 What's the child father's completed educational level?

孩子父亲的学历

[faedu]

None 没有.....1. ☐

Primary 小学 .....2. ☐

Secondary 中学.....3. ☐

“O” / “N” Levels “O” / “N” 水准 .....4. ☐

“A” Levels / Polytechnic / Diploma / ITE / Certificate

“A” 水准 / 理工学院 / 学位文凭 / 工艺教育学院 / 文凭

.....5. ☐

University education (degree and above, including bachelor, master and PhD)

大学教育 (学位及以上，包括学士、硕士和博士).....6. ☐

Others 其它.....7. ☐

Specify: _____

请说明：

RF 拒绝回答.....98. ☐

DK 不知道.....99. ☐

[faedspf]

A.5 What’s the child mother’s completed educational level?

孩子母亲的学历

[moedu]

None 没有.....1. ☐

Primary 小学.....2. ☐

Secondary 中学.....3. ☐

“O” / “N” Levels “O” / “N” 水准 .....4. ☐

“A” Levels / Polytechnic / Diploma / ITE / Certificate

[moedspf]

"A" 水准 / 理工学院 / 学位文凭 / 工艺教育学院 / 文凭

.....5. ☐

University education (degree and above, including bachelor, master and PhD)

大学教育 (学位及以上，包括学士、硕士和博士).....6. ☐

Others 其它.....7. ☐

Specify: _____

请说明

RF 拒绝回答.....98. ☐

DK 不知道.....99. ☐

SECTION B: Mother's Smoking History.

B 项: 孩子母亲吸烟的历史

B1 Has the mother ever smoked?

(At least one cigarette a day for 1 year or longer)

您可曾吸烟?

[mosmoke]

NO 没有.....**(SKIP TO 到 C 1)**..... 0. ☐

YES, and she currently still smokes

有，目前还在吸烟

(每天至少吸一支香烟有整年或更长的时间)

.....**(SKIP TO 到 B 3)**.....	1. ☐
YES, but she quit smoking 有，目前已戒烟	
.....**(GO TO 到 B 2)**.....	2. ☐
RF 拒绝回答.....	98. ☐
DK 不知道.....	99. ☐

B2 If the mother quit smoking, how long ago did she quit? [moquit]

如果已戒烟，那是多久的事?

⌊_⌋⌊_⌋ .....YEARS AGO 年前	
RF 拒绝回答.....	98. ☐
DK 不知道.....	99. ☐

B3 At what age did the mother start smoking cigarettes on a regular basis? [mosmkage]

您几岁开始有规律性地吸烟?

⌊_⌋⌊_⌋ .....YEARS OLD 岁	
RF 拒绝回答.....	98. ☐
DK 不知道.....	99. ☐

B4 If the mother smokes / used to smoke manufactured cigarettes, what is the number of cigarettes that he smoked per day? [mosmkno]

如果您是吸烟者/ 习惯吸香烟厂的香烟，一天吸多少支香烟?

6 cigarettes or less 6 支香烟或更少	1. ☐
7 – 12 cigarettes 7 – 12 支香烟.....	2. ☐
13 – 22 cigarettes 13 – 22 支香烟.....	3. ☐
23 – 32 cigarettes 23 – 32 支香烟.....	4. ☐
33 – 42 cigarettes 33 – 42 支香烟.....	5. ☐
43 cigarettes or more	

43 支香烟或更多..... 6. ☐

RF 拒绝回答..... .....98. ☐

DK 不知道.....99. ☐

SECTION C: Father's Smoking History.

C 项: 孩子父亲吸烟的历史

C1 Has the father ever smoked?

(At least one cigarette a day for 1 year or longer)

[fasmoke]

您可曾吸烟?

(每天至少吸一支香烟有整年或更长的时间)

NO 没有..... **(SKIP TO 到 D)**..... 0. ☐

YES, and he currently still smokes

有，目前还在吸烟

.....**(SKIP TO 到 C 3)**..... 1. ☐

YES, but he quit smoking

有，目前已戒烟

.....**(GO TO 到 C 2)** ..... 2. ☐

RF 拒绝回答..... .....98. ☐

DK 不知道..... .....99. ☐

C2 If the father quit smoking, how long ago did he quit?

[faquit]

如果已戒烟，那是多久的事?

└┴┘ .....YEARS AGO 年前

RF 拒绝回答..... .....98. ☐

DK 不知道..... .....99. ☐

C3 At what age did the father start smoking cigarettes on a regular basis?

[fasmkage]

└┴┘ .....YEARS OLD 岁

您几岁开始有规律性地吸烟?

RF 拒绝回答..... .....98. ☐

DK 不知道..... .....99. ☐

C4 If the father smokes / used to smoke manufactured cigarettes, what is the number of cigarettes that he smoked per day? [fasmkno]

如果您是吸烟者/ 习惯吸香烟厂的香烟，一天吸多少支香烟?

6 cigarettes or less

6 支香烟或更少 ..... 1. ☐

7 – 12 cigarettes 7 – 12 支香烟..... 2. ☐

13 – 22 cigarettes 13 – 22 支香烟 ..... 3. ☐

23 – 32 cigarettes 23 – 32 支香烟 ..... 4. ☐

33 – 42 cigarettes 33 – 42 支香烟 ..... 5. ☐

43 cigarettes or more

43 支香烟或更多..... 6. ☐

RF 拒绝回答..... .....98. ☐

DK 不知道.....99. ☐

SECTION D: Contact Details

D 项: 联络方式 This section can be completed by the respondent
(这个部分可以由受访者完成)

		Home Tel 住家电话	Office Tel 办公室电话	Pager no 传呼机	Hand-phone 手提电话	Email 电子邮件
D.1. [faname]	Father's name: 父亲的名字: _____	[fatelh]	[fatelo]	[fapager]	[fahp]	[faemail]
D.2. [moname]	Mother's name: 母亲的名字 _____	[motelh]	[motelo]	[mopager]	[mohp]	[moemail]
D.3. [carename]	Other Care Giver 其他看护人 (Please Specify) （请说明） _____	[telhcl]	[telocl]	[pagercl]	[hpcl]	[emaicl]

1. Other Care Giver may be grandparents, baby sitter, maid etc. (其他看护者可以是祖父母，保姆，佣人)

E. **This section can be completed by the respondent.** (这个部分可以由受访者完成)

Now I'd like to get the names, addresses, and telephone numbers of one person who does not live with you now but who would know how to reach you if you move. Anyone we contact would be asked only if they know how to reach you. They won't be asked anything else, and they won't be given any information about you. Please try to include at least one close relative/friend who lives in the Singapore but does not live with you.

请提供至少一个不与您居住在一起，但居住在新加坡并时常与您保持联络的亲戚/朋友的联络资料。如果您搬家，我们将会联络他，而唯一的目的就是为了联络你，我们将不会问他其它的问题。

[crname] 1. FULL NAME 全名:

_____

[crrelate] 2. PERSON'S RELATIONSHIP TO YOU 你们两人的关系:

_____

[cradd] 3. PRESENT ADDRESS 现在的住址:

_____

[crpcode] _____S( )

[crtelh] 4. HOME TELEPHONE NUMBER 住家电话 .....└┴┴┴┘└┴┴┴┘

[crtelo] 5. WORK TELEPHONE NUMBER 办公室电话 .....└┴┴┴┘└┴┴┴┘

[crtelhp] 6. HANDPHONE NUMBER 手提电话 .....└┴┴┴┘└┴┴┴┘

Family History (Immediate Family: Father, Mother, and Children) of wearing glasses, contact lens and eye related diseases

家庭背景(直属亲人：父亲，母亲和孩子) 有 戴眼镜，隐形眼镜和有关眼睛的疾病

(1) Name 姓名	(2) Relationship 关系	(3) Gender 性别 1 = Male 男 2 = Female 女	(4) History of wearing glasses/contact lens/laser surgery 是否曾配戴眼镜或隐形眼镜/激光手术 1 = Yes 是 0 = NO 不是	(4.1) If Yes for previous question (who had a history of wearing any glasses/contact lens) 如果曾配戴眼镜或隐形眼镜，请回答以下问题		(5) Do you have any of the following eye diseases? 是否患上以下眼疾？		Child participating in the study 孩子是否参与这项研究 1 = Yes 是 0 = No 不是	If the child is participating in the study, please provide child study ID 如果孩子参与这项研究，请提供他的研究编号。
				(4.1.1) Age first wore glasses (yrs) 一开始配戴眼镜的年龄（岁数）	(4.1.2) Glasses are for 眼镜是用来： 1 = Distance 远距离 2 = Close 近距离 3 = Both Distance and close 远和近距离 4 = Contact Lens 隐形眼镜 5 = Astigmatism 散光	(5.1) **Amblyopia 弱视** 1 =Yes 是 0 = No 不是	(5.2) **Strabismus 斜视** 1 = Yes 是 0 = No 不是		
[faname]	**Father 父亲**	**1** [fagender]	[glassfa]	[glagefa]	[gltypfa]	[ambfa]	[strabfa]	**NA**	**NA**
[moname]	**Mother 母亲**	**2** [mogender]	[glassmo]	[glagemo]	[gltypmo]	[ambmo]	[strabmo]	**NA**	**NA**

Name of child(ren):

[sib1nam]	Child1 孩子 1	[sibgen1]	[glassib1]	[glagesb1]	[gltypsb1]	[ambsb1]	[strabsb1]	[sibptp1]	[sibid1]
[sib2nam]	Child2 孩子 2	[sibgen2]	[glassib2]	[glagesb2]	[gltypsb2]	[ambsb2]	[strabsb2]	[sibptp2]	[sibid2]
[sib3nam]	Child3 孩子 3	[sibgen3]	[glassib3]	[glagesb3]	[gltypsb3]	[ambsb3]	[strabsb3]	[sibptp3]	[sibid3]
[sib4nam]	Child4 孩子 4	[sibgen4]	[glassib4]	[glagesb4]	[gltypsb4]	[ambsb4]	[strabsb4]	[sibptp4]	[sibid4]
[sib5nam]	Child 5 孩子 5	[sibgen5]	[glassib5]	[glagesb5]	[gltypsb5]	[ambsb5]	[strabsb5]	[sibptp5]	[sibid5]

1. **Amblyopia: Amblyopia is poor vision in an eye that can not be corrected with glasses or contact lenses and the eye looks normal.**
   弱视是一种视力的缺陷，不能即由眼镜或隐性眼镜而矫正，而眼睛也看似正常。

2. **Myopia: Myopia or nearsightedness or needs to wear glasses to see far away.**
   近视是需要戴眼镜才能看清远距离的东西。

3. **Strabismus is when the eyes are not properly lined – up. This happens when one eye looks straight ahead and the other eye crosses in or wanders out.**
   斜视 – 两只眼睛不能完全地列好，即当一只眼睛看向前面但另一只眼睛交叉或目光无目的地移动。

A Study on Strabismus, Amblyopia and Refractive Error in Singapore (STARS) Preschoolers

Sticky Label with following info:

1. Study No.[sno]
2. Name
3. BC number
4. Age

_____

ME: [timea] _____

(24-hr system)

	Tick when Completed	Investigator Code	Time Completed
1. Registration 2. Consent	☐ ☐	☐☐ ☐☐	☐☐:☐☐
3. Test for Glasses 4. Stereopsis / Randot Preschool Stereoacuity Test 5. Eye Alignment 6. Ductions / Versions 7. Bruckner Test: 8. Fixation Preference Test 9. Colour Vision 10. Visual Acuity (With & without Glasses) 11. Test for Pupil 12. Anterior Segment Evaluation	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	☐☐ ☐☐ ☐☐ ☐☐ ☐☐ ☐☐ ☐☐ ☐☐ ☐☐ ☐☐	☐☐:☐☐
13. Eye Drops	☐	☐☐	☐☐:☐☐
14. Measure Weight, Length and Height	☐	☐☐	☐☐:☐☐

15. Measure BP and Skin Fold			
16. Interview	☐	☐☐	☐☐:☐☐
17. Axial length 18. Auto-refraction 19. Retinoscopy	☐ ☐ ☐	☐☐ ☐☐ ☐☐	☐☐:☐☐
19. Visual Acuity (Same-day) Retest	☐	☐☐	☐☐:☐☐
20. Fundus Evaluation 21. Diagnosis	☐ ☐	☐☐ ☐☐	☐☐:☐☐
22. Sub-Studies	☐	☐☐	☐☐:☐☐
23. Case File Completed/Checked	☐	☐☐	☐☐:☐☐

CLINICAL EXAMINATION: SHORT FORM

(1) Glasses: [gl] **Yes** 1. ☐ **RX: OD** _____sph _____cyl ___axis **OS** _____sph _____cyl ___axis

N0 2. ☐ **[glsphr]** **[glcylr]** **[glaxr]** **[glsphl]** **[glcyll]** **[glaxl]**

(2) Stereopsis: [stereo]

	800”	400”	200”	100”	60”	40”	No stereopsis	Unable	N/A
Randot Preschool	☐	☐	☐	☐	☐	☐	☐	☐	☐
Stereoacuity Test	1	2	3	4	5	6	7	8	9

(aged 30 months or older only)

(3) Accommodative lag [acclag] 1☐ **Applicable** 0☐ **Not Applicable**

(≥ 42 months)

1. Eye **[aclag]** ☐ 1 Right ☐ 2 Left ☐ 3 Unable

2. Glasses **[acglas]** ☐ 1 with ☐ 2 without ☐ 3 Unable

3. Lag

variable name		lag	RE	LE
lagr1	**lagl1**	1		
lagr2	**lagl2**	2		
lagr3	**lagl3**	3		

(4) Bruckner Test:

(Red Reflex)

[redrfl]

Symmetry ……1. ☐

Asymmetry……...2. ☐

Unable ……..3. ☐

(4.1) Nystagmus : **[nstg]**

Present ……..1. ☐

Absent ……...2. ☐

If present, type:
[nstgyp]_____

(5) Eye Alignment (UCT): [align] Non-Strabismic 1. ☐ Strabismic 2. ☐ Can't determine 3. ☐

If Strabismic, tick the abnormalities present (5.1 to 5.6):

Items	1. Distance		2. Near	
	With correction	**Without correction**	**With correction**	**Without correction**
5.1 frequency	Constant 1. ☐ Intermittent 2. ☐ **[dcfr]**	Constant 1. ☐ Intermittent 2. ☐ **[dwcfr]**	Constant 1. ☐ Intermittent 2. ☐ **[ncfr]**	Constant 1. ☐ Intermittent 2. ☐ **[nwcfr]**
5.2 Laterality	RE 1. ☐ LE 2. ☐ alt 3. ☐ **[dclt]**	RE 1. ☐ LE 2. ☐ alt 3. ☐ **[dwclt]**	RE 1. ☐ LE 2. ☐ alt 3. ☐ **[nclt]**	RE 1. ☐ LE 2. ☐ alt 3. ☐ **[nwclt]**
5.3 Horizontal Direction	ET 1. ☐ XT 2. ☐ **[dchd]**	ET 1. ☐ XT 2. ☐ **[dwchd]**	ET 1. ☐ XT 2. ☐ **[nchd]**	ET 1. ☐ XT 2. ☐ **[nwchd]**
5.4 Vertical Direction	**Hyper T:** RE 1. ☐ LE 2. ☐ **[dcvd]**	**Hyper T:** RE 1. ☐ LE 2. ☐ **[dwcvd]**	**Hyper T:** RE 1. ☐ LE 2. ☐ **[ncvd]**	**Hyper T:** RE 1. ☐ LE 2. ☐ **[nwcvd]**
5.5. Alternate Prism Cover Test 1. Horizontal magnitude	(1) _____ pd Unable (99) ☐	(1) _____ pd Unable (99) ☐	(1) _____ pd Unable (99) ☐	(1) _____ pd Unable (99) ☐

2. Vertical magnitude	**[dchm]** (1) _____ pd Unable (99) ☐ **[dcvm]**	**[dwchm]** (1) _____ pd Unable (99) ☐ **[dwcvm]**	**[nchm]** (1) _____ pd Unable (99) ☐ **[ncvm]**	**[nwchm]** (1) _____ pd Unable (99) ☐ **[nwcvm]**
5.6 Dissociate Vertical Deviation (DVD) **[dvd1]**	Yes 1. ☐ No 2. ☐ If "Yes" please specify the effected eyes: **[dvdeye1]** RE 1. ☐ LE 2. ☐ BE 3. ☐			

(6) Ductions/Versions: [duction]

All Normal 1. ☐

Abnormality present 2. ☐

*** If abnormality presence; please fill in the following: (6.1 to 6.6)**

Muscle	**(1) RE**	**(2) LE**
6.1 Superior Oblique **(In and Down)**	Over Action 1. ☐ **[so_rt]** Under Action 2. ☐ **[so_rt1]**	Over Action 1. ☐ **[so_lt]** Under Action 2. ☐ **[so_lt1]**
6.2 Inferior Oblique **(In & Up)**	Over Action 1. ☐ **[io_rt]** Under Action 2. ☐ **[io_rt1]**	Over Action 1. ☐ **[io_lt]** Under Action 2. ☐ **[io_lt1]**

6.3 Superior Rectus **(Out & Up)**	Over Action 1. ☐ **[sr_rt]** Under Action 2. ☐ **[sr_rt1]**	Over Action 1. ☐ **[sr_lt]** Under Action 2. ☐ **[sr_lt1]**
6.4 Inferior Rectus **(Out and Down)**	Over Action 1. ☐ **[ir_rt]** Under Action 2. ☐ **[ir_rt1]**	Over Action 1. ☐ **[ir_lt]** Under Action 2. ☐ **[ir_lt1]**
6.5 Medial Rectus **(In)**	Over Action 1. ☐ **[mr_rt]** Under Action 2. ☐ **[mr_rt1]**	Over Action 1. ☐ **[mr_lt]** Under Action 2. ☐ **[mr_lt1]**
6.6 Lateral Rectus **(Out)**	Over Action 1. ☐ **[lr_rt]** Under Action 2. ☐ **[lr_rt1]**	Over Action 1. ☐ **[lr_lt]** Under Action 2. ☐ **[lr_lt1]**

**possible answers are 0, 1, 2, 3, 4, or unable (9).*

GRADE OF FIXATION PREFERENCE:

(7) Fixation Preference

test: [prefer]

(Place 12△ base-down loose prism in front of the Rt eye – observe the response, then repeat this step to the Lt eye)

Alternates	Holds well	Holds fair (1-3 sec)	No Hold (< 1 sec)	Unable
A (1)	B (2)	C (3)	D (4)	E (5)

(1) RE Preference ☐

(2) LE Preference ☐

(3) NO Preference ☐ **[eprefer]**

(8) Color Vision: [cv] 1☐ Applicable 0☐ Not Applicable

(30 months or older only)

Ishihara at 40 cm (Per Eye):

Indicate only numbers that are not given by normal person response in RE / LE column, respectively.

RE	LE	Plate	Normal Response	R-G Deficiencies Responses	RE	LE
ihhr1	ihhl1	1	12	12		
ihhr2	ihhl2	2	8	3		
ihhr3	ihhl3	3	29	70		
ihhr4	ihhl4	4	5	2		
ihhr5	ihhl5	5	3	5		
ihhr6	ihhl6	6	15	17		
ihhr7	ihhl7	7	74	21		

ihhr8	ihhl8	8	6	X					
ihhr9	ihhl9	9	45	X					
ihhr10	ihhl10	10	5	X					
ihhr11	ihhl11	11	7	X					
ihhr12	ihhl12	12	16	X					
ihhr13	ihhl13	13	73	X					
ihhr14	ihhl14	14	X	5					
ihhr15	ihhl15	15	X	45					
				Protan		Deutan			
				Strong	Mild	Strong	Mild		
ihhr16	ihhl16	16	26	6	(2) 6	2	2 (6)		
ihhr17	ihhl17	17	42	2	(4) 2	4	4 (2)		

Note:

- The mark 'X' shows that the plate cannot be read. **Data key in as "100"**
- The numerals in '( )' show that they can be read but they are comparatively unclear.
- Number of errors allowed to consider no colour deficiencies = 5 errors
- The mark **'√'** shows that the plate can be read, Data key in as "888"

1. Right: [cvr]	**2. Left:** [cvl]
Normal 1. ☐	Normal 1. ☐
Abnormal 2. ☐	Abnormal 2. ☐
Unable 3. ☐	Unable 3. ☐
Please specify: **[cvrspf]**_____)	Please specify: (**[cvlspf]**_____)

(9) Visual Acuity [va] 1 ☐ **Applicable** 0 ☐ **Not Applicable**

(30 months or older only):

1. Without Glasses :

VR [var]_____ **VL [val]**_____

Unable 99 ☐ Unable 99 ☐

2. With Glasses (if any):

VR [glvar]_____ **VL[glval]**_____

Unable 99 ☐ Unable 99 ☐

(10) Pupils:

[pupil]

(1) ☐ Normal

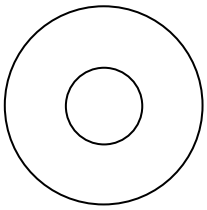
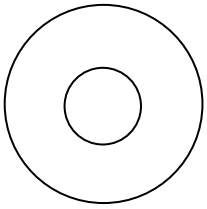
(2) ☐ Afferent pupillary defect (OD)

(3) ☐ Afferent pupillary defect (OS)

(4) ☐ Other. Please specify**: [pupilo]**

_____

(11) Anterior segment evaluation :

	(RE)	(LE)
		
	9.1 (Right) [as_rt]	**9.2 (Left) [as_lt]**
Normal	**1.** ☐	**Normal** **1.** ☐
Abnormal	**2.** ☐	**Abnormal** **2.** ☐

For abnormal finding(s), please complete the followings:

	1. Yes	2. No		1. Yes	2. No
11.3. Ptosis **[rptosis]**	☐	☐	**[lptosis]**	☐	☐
11.4 Cataract **[rcat]**	☐	☐	**[lcat]**	☐	☐
11.5 Epiblepharon **[repiblep]**	☐	☐	**[lepiblep]**	☐	☐
11.6 Others **[rasoths]**	☐	☐	**[lasoths]**	☐	☐

(Please specify):

_____	_____
[rasspf]	**[lasspf]**
_____	_____

(12) OCULAR DOMINANCE [od] 1☐ Applicable 0☐ Not Applicable

≥ 48 months and above **2☐ Applicable but Unable**

> **non-cyclopleged eye**

1. "Hole in the card" Test

_____

Right Eye- Object seen through the hole ☐1Yes ☐0 No

(Left eye covered) **[odr1]**

Left Eye- Object seen through the hole ☐1Yes ☐0 No

(Right eye covered) **[odl1]**

2. "Hole in the card" Test

_____

Right Eye- Object seen through the hole ☐1Yes ☐0 No

(Left eye covered) **[odr2]**

Left Eye- Object seen through the hole ☐1Yes ☐0 No

(Right eye covered) **[odl2]**

3. "Hole in the card" Test

_____

Right Eye- Object seen through the hole ☐1Yes ☐0 No

(Left eye covered) **[odr3]**

Left Eye- Object seen through the hole ☐1Yes ☐0 No

(Right eye covered) **[odl3]**

4. “Tube” Test **[odtube]** ☐1Right eye ☐2 Left Eye

5. Observation

1. Hand used for drawing\coloring ☐1Right hand ☐2 Left hand

[drawing]

2. Hand used for picking up a toy ☐1Right hand ☐2 Left hand

[picking]

(13) Eyedrops:

0.5% proparacaine @ _____H _____

1.0% cyclopentolate (0.5% if child ≤ 1 year) @ _____H _____

2.5% pheynyephrine **[mydfrin]** @ _____H _____

(5 minutes later administer)

↓

1.0% cyclopentolate (0.5 % if child ≤ 1 year) @ _____H _____

(5 minutes later administer)

↓

1.0% cyclopentolate (0.5 % if child ≤ 1 year) @ _____H _____

Comment: _____

[comeye]

Total number of cyclopentolate eyedrop installed **[eyedrno]:** ☐

**possible answers are 0, 1, 2, 3*

(14) IOL Master **(Biometry) [iol]** **(30 months or older only)**	1. ☐ Done 2. ☐ Refused 3 ☐ Unable 4. ☐ Not Applicable Comments **[iolcom]** _____ __ **(Staple IOLMaster paper here and write the child's name)**

(15) REFRACTION: **[auto]**

(30 minutes after last drop of cyclopentolate)

1. ☐ Done 2. ☐ Refused 3. ☐ Unable 4. ☐ Not Applicable

Comments **[comauto]**

A. Autorefraction (for 24 months or older)

Retinomax : **[retinomax]** 1 ☐ **Applicable** 0 ☐ **Not Applicable** 2. ☐ **Unable**

B. Retinomax (for less than 24 months)

Check the following:

- Ensure best readings.
- Cross – out readings with * and extra readings in excess of 5.
- Retake if more than 1 * or SD > ± 0.25.

(Staple paper here and write the child's name)

	OD _____ OS_____
(16) <u>Retinoscopy:</u> **(If autorefraction/ retinomax fails or if no cyclo done)**	**[retiscopy]** 1☐ **Applicable** 0☐ **Not Applicable** 2. ☐ **Unable** **[stsphr] [stcylr] [staxr]** **[stsphl] [stcyll] [staxl]** OD: _____sph_____cyl_____axis OS: _____sph_____cyl_____axis

(17) Visual Acuity Re-Testing:

VA RETEST CRITERIA

Criteria Set #1		Criteria Se t#2
1. 20/30 or worse (or unable) in one eye AND 2. ≥ 2 line IOD AND 3. Unilateral or bilateral amblyogenic factor*	or	VA decrease: a. <4 years of age: 20/60 or worse (or unable) in one or both eyes b. ≥4 years of age: 20/50 or worse (or unable) in one or both eyes

Amblyogenic Factors

Unilateral	Bilateral
1. Anisometropia (*spherical equivalent): ➢ 1.00D* Hyperopic anisometropia ➢ 3.00D* Myopic anisometropia ➢ 1.50D* Astigmatic anisometropia 2. Antimetropia (*spherical equivalent): Eye with the greater refractive error has: ≥1.00D* of Hyperopia or ≥3.00D* of Myopia 3. History of physical obstruction along the line of sight of one eye 4. Strabismus in primary gaze at distance and/or near fixation or a history of strabismus surgery (or botulinium)	1. Isometropia: ≥4.00D Hyperopia ≥6.00D Myopia ≥2.50D Astigmatism 2. History of physical obstruction along the line of sight in both eyes

Same-day retest (Cyclopleged)

OD: _____sph _____cyl _____axis VA _____

Unable(99) ☐

[resphr] [recylr] [reaxr] **[revar]**

OS: _____sph _____cyl _____axis VA _____

Unable(99) ☐

[resphl] [recyll] [reaxl] **[reval]**

[return] Passed Criteria ☐ 1

For Return-Visit Retest (Non-cyclopleged) ☐ 2

(18) Blood Pressure and Skin Fold: [BP] 1☐ Applicable 0☐ Not Applicable

1. **48 months and above** 2☐ **Applicable but Refused**
2. **BP with 1 minute interval**

BLOOD PRESSURE PULSE RATE

Systolic *Diastolic*

{bpsys1} {bpdia1} 1ST Reading ☐☐☐/☐☐☐mmHg **{bppul1}**☐☐☐ beats/min

{bpsys2} {bpdia2} 2ND Reading ☐☐☐/☐☐☐mmHg **{bppul2}**☐☐☐ beats/min

{method12} Measuring method ☐0 Dinamap ☐1 Manual ☐2 Omron

Note:

If the difference between the 2 readings are **greater than** 10mmHg **SBP** and / or 5mmHg **DBP**, take a 3rd reading. Accept the two closest readings for data entry.

{bpsys3} {bpdia3} 3RD Reading ☐☐☐/☐☐☐mmHg **{bppul3}**☐☐☐ beats/min

{method3} Measuring method ☐0 Dinamap ☐1 Manual ☐2 Omron

SKIN FOLD (mm) [sf] 1☐ **Applicable** 0☐ **Not Applicable** 2☐ **Applicable but Refused**

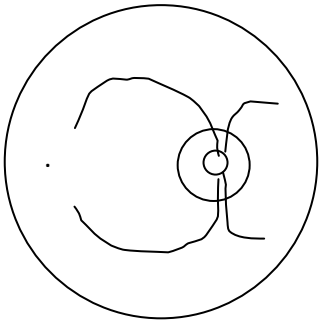
0

1. ☐☐.☐ [skfo1] **2.** ☐☐.☐ [skfo2] **3.** ☐☐.☐ [skfo3]

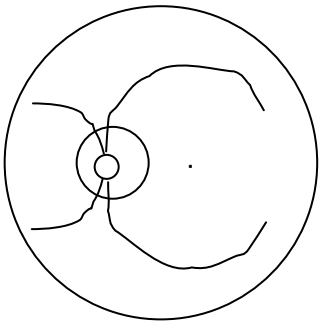
(19) Height (cm) (24 months or older) [htcm] **Or** **(20) Length (cm) (if < 24 months):** [ltcm]	☐☐☐.☐ cm ☐☐☐.☐ cm

(21) Weight (kg): [wtchild] [wtparent] [wtcomb]	21.1. Weight of child (only) = ☐☐.☐ (kg) **If child < 24 months** 21.2. Weight of **parent** = ☐☐.☐ (kg) 21.3. Weight of **child and parent** = ☐☐.☐ (kg)

(22) Fundus:



(RE)



(LE)

	1. Normal	2.Abnormal	3. Unable	1. Normal	2.Abnormal	3. Unable
1.Macular	☐	☐	☐	☐	☐	☐
[macular]				**[macular]**		
2. Disc	☐	☐	☐	☐	☐	☐
[discr]				**[discl]**		
3. Media	☐	☐	☐	☐	☐	☐
[mediar]				**[medial]**		
4.Posterior pole of retina	☐	☐	☐	☐	☐	☐
[postretr]				[postretl]		
5.Peripheral retina	☐	☐	☐	☐	☐	☐
[periretr]				**[periretl]**		
6. Describe lesions:						
[desbr]	_____			**[desbl]**	_____	

[comfun] _____

OTHER TEST

1. Fundus Photo **[funpto]** Taken ☐ **1** Not Taken ☐ **0** Not Applicable ☐ **2**

(>47 months)

2. ORA **[ora]** Done ☐ **1** Not Done ☐ **0** Not Applicable ☐ **2**

3. DNA/Saliva **[dna]** Collected ☐ **1** Not Collected ☐ **0**

4.. Peripheral Refraction **[prp]** Done ☐ **1** Not Applicable ☐ **2** Unable ☐ **99**

(≥48 months, right eye only, after cycloplegia)

23. DIAGNOSIS: Please (✔) the appropriate clinical diagnosis and their sub-clinical diagnosis

1. **Amblyopia and Decreased Visual Acuity** .....Yes 1. ☐
[dxamdva] No 2. ☐
Unclassified 3. ☐

*** If it is "Amblyopia and Decreased Visual Acuity", please (✔) the appropriate**

[amdva]

1.1. Unilateral Amblyopia .....1. ☐

1.2. Suspected Unilateral Amblyopia ..... 2. ☐

1.3 Suspected Bilateral Amblyopia ..... 3. ☐

1.4. Unilateral Decreased Visual Acuity; Not Amblyopia ..... 4. ☐

1.5. Bilateral Amblyopia ..... 5. ☐

1.6. Bilateral Decreased Visual Acuity; Not Amblyopia ..... 6. ☐

1.7. Normal Visual Acuity ..... 7. ☐

2. **Amblyopia** .....Yes 1. ☐

[dxambly] No. 2. ☐

If it is "Amblyopia", please (✔) the appropriate diagnosis

[ambly]

(1) Strabismic amblyopia ..... 1. ☐

(2) Anisometropic amblyopia ..... …2. ☐

(3) Combined strabismic/ anisometropic amblyopia .....3. ☐

(4) Isoametropic amblyopia .....4. ☐

(5) Deprivation amblyopia ....5. ☐

3. **Strabismus** .....Yes 1. ☐

[dxstrab] No. 2. ☐ **(Skip to 4)**

If it is "Strabismus", please (✔) the appropriate diagnosis.

3.1. **Esotropia** .....Yes 1. ☐

[esotrop] No 2. ☐

[esotype] If "yes" to # (3.1), **please (✔) one of the followings:**

3.1.1. Esotropia, Refractive Accommodative .....1. ☐

3.1.1.a Partially Accommodative.....a. ☐ [18]

3.1.1.b Complete Accommodative .....b. ☐ [19]

3.1.2. Esotropia, Non- Refractive Accommodative .....2. ☐

3.1.3. Esotropia, Mixed Accommodative .....3. ☐

3.1.4. Esotropia, Non-Accommodative (Basic) .....4. ☐

3.1.5. Sensory Esotropia ..... .5 ☐

3.1.6. Esotropia, Non-comitant .....6. ☐

3.1.7. Infantile Esotropia Syndrome ..... 7. ☐

3.2. **Exotropia** .....Yes 1.☐

[dxexop] No 2. ☐

If it is **"Exotropia"**, **please (✔) one of the followings:**

[exotrop]

3.2.1. Intermittent Exotropia .....1. ☐

3.2.2. Constant Exotropia .....2. ☐

3.2.3. Sensory Exotropia .....3. ☐

3.2.4. Exotropia, Non-comitant ..... 4. ☐

[exospf] (Please Specify type _____)

3.3. **Hypertropia** .....Yes 1. ☐

[hyptrop] No 2. ☐

3.4. **Microtropia** .....Yes 1. ☐

[mictrop] No 2. ☐

3.5. **DVD** .....Yes 1. ☐

[dxdvd] No 2. ☐

If it is **"DVD"**, **please (✔) one of the followings:**

[dvdeye]

3.5.1. Right Eye.....1. ☐

3.5.2 Left Eye.....2. ☐

3.5.3 Both Eye..... .....3. ☐

4. **Nystagmus** ..... Yes 1. ☐
[dxnyst] No. 2. ☐

If it is **"Nystagmus"** , **please (✔) one of the followings:**

[nystyp]

5.1. Manifest.....1. ☐

5.2. Latent ……..2. ☐

5. **Any other clinical Diagnosis** ……Yes 1. ☐
[othdx] No 2. ☐

If "yes" please specify. **[othdxspf]**

_____-

Finish Time: _____ **[timef]**

(24-hr system)

RECRUITMENT VISIT
ELIGIBILITY QUESTIONNAIRE

Study ID : _____ Date of interview : _____

Interviewer code : _____ Interview start time : _____

Date of Birth: ☐☐ ☐☐ ☐☐☐☐ Age: ☐☐ years
D D M M Y Y Y Y

Status: ☐ 1. Singapore citizen ☐ 2. Singapore PR ☐ 3. Foreigner

Address:

Block/House no/Building Name/Street: _____

Unit no:_____ Postal Code: _____

Do you intend to reside in Singapore for the next 5 years? ☐ 0: No ☐ 1: Yes

Do you intend to deliver in KKH or NUH? ☐ 0: No ☐ 1: Yes

Do you intend to donate cord, cord blood and placenta?

☐ 0: No ☐ 1: Yes ☐ 99: Don't know

Race:

Baby's mother

☐ 1: Chinese ☐ 2: Malay ☐ 3: Indian ☐ 4 : Others

Baby's maternal grandfather (mother's father)

☐ 1: Chinese ☐ 2: Malay ☐ 3: Indian ☐ 4: Others

Baby's maternal grandmother (mother's mother)

☐ 1: Chinese ☐ 2: Malay ☐ 3: Indian ☐ 4: Others

Baby's father

☐ 1: Chinese ☐ 2: Malay ☐ 3: Indian ☐ 4: Others

Baby's paternal grandfather (father's father)

☐ 1: Chinese ☐ 2: Malay ☐ 3: Indian ☐ 4: Others

Baby's paternal grandmother (father's mother)

☐ 1: Chinese ☐ 2: Malay ☐ 3: Indian ☐ 4: Others

Are the grandparents of the baby of homogenous ethnicity?

☐ 0: No

☐ 1: Yes

1. OBSTETRIC HISTORY:

1.1. When was the first day of your last menstrual period (LMP)?

☐☐ D D ☐☐ M M ☐☐☐☐ Y Y Y Y ☐ 99: Don't know

1.2. How many weeks is your pregnancy (based on LMP)?

☐☐ weeks

1.3. Is this current pregnancy conceived through IVF?

☐ 0: No ***go to question 2.1***

1: Yes ***Please refer to IVF study team***

1.4. Have you enrolled for an IVF study?

☐ 0: No

1: Yes

2. CANCER

2.1. Have you ever been told by a doctor that you have cancer? 0:

☐ No ***go to question 3.1***

1: Yes

2.2. When did the doctor first tell you that you had cancer? *(Fill in one of the options below)*

☐☐ Age ☐☐☐☐ Year ☐ 99: Don't know

2.3. Are you currently on chemotherapy?

☐ 0: No

1: Yes

3. MENTAL ILLNESSES

3.1. Have you ever been told by a doctor that you are suffering from a mental or psychological condition?

☐ 0: No ***go to question 4.1***

1: Yes, please specify: _____

3.2. When the diagnosis was first made? ***(Fill in one of the options below)***

Age ☐☐ (or) Year ☐☐☐☐

3.3. Are you currently taking any medication for mental or psychological condition?

0: No

1: Yes, please specify: _____

4. OTHER LONG TERM ILLNESSES

4.1. Have you ever been told by a doctor that you have other long term illnesses?

0: No ***go to question 5.1***

1: Yes

If yes, please specify:

S/N	Type of illness	Age (yr)	OR	Year (YYYY)	99:Don't know
1	Type 1 diabetes		OR		
If others, please specify:					
			OR		
			OR		
			OR		

5. MEDICATION

5.1. In the past year (before this pregnancy), did you take any regular medications, supplements and/or traditional medicine?

0: No ***End of eligibility questionnaire***

1: Yes

ELIGIBILITY: ☐ **0: NO** ☐ **1: YES**

Interview end time: _____

THANK YOU VERY MUCH FOR YOUR TIME !

RECRUITMENT VISIT

1ST CLINIC VISIT QUESTIONNAIRE

Study ID : _____ Date of interview : _____

Interviewer code : _____ Interview start time : _____

1. DEMOGRAPHY

I would like to start by asking you some questions about yourself.

1.1. How old were you when you left long term full time education?
(enter current age if still studying) ☐☐ years

1.5. What is the highest level of education that you have attained?

☐ 0: None
1: Primary (PSLE)
2: Secondary (GCE 'O'/ 'N' levels)
3: ITE/NTC
4: GCE 'A' levels/Polytechnic/diploma
5: University
6: Others, specify:_____

1.6. What is your marital status?

☐ 0: Single and living with the baby's father
1: Single and **not** living with the baby's father
2: Married (living with husband)
3: Married but **not** living with husband
4: Separated
5: Divorced
6: Widowed
7: Others, specify:_____

1.7. What is your religion?

☐ 0: No religion
1: Buddhism
2: Christianity
3: Islam
4: Taoism
5: Hinduism
6: Others, specify:_____

1.8. Where were you born?

☐ 1: Singapore ***go to question 2.1***
2 Malaysia
3 China
4 India
5 Others, specify:_____

1.6. When did you move to Singapore?

M M Y Y Y Y

2. OCCUPATION

2.1. What is your current job?

☐ 1: Legislator/senior official
2: Professional
3: Technician & associated professional
4: Clerical worker
5: Service worker
6: Agricultural worker
7: Production craftsman
8: Plant and machine operator
9: Homemaker
10: Retired
11: Student
12: Unemployed
13: Others, specify:_____
14: Refused

3. HOUSING AND HOUSEHOLD COMPOSITION

3.1. What type of accommodation do you live in?

☐ 1: 1-2 room HDB flat
2: 3 room HDB flat
3: 4-5 room HDB flat
4: HUDC/executive flat
5: Condominium
6: Landed property
7: Others, specify:_____

3.2. Does anyone else live together with you?

☐ 0: No ***go to question 4.1***
1: Yes ***please specify in the following table (next page)***

For each person living in the household (apart from the woman herself), complete one line.
A household is defined as a group of people who share a living room or eat together for at least one meal a day.

CHILDREN

*For **all children**, record date of birth (or age if D.O.B. not available).*
*For the **woman's own children**, give the child's birth weight.*

S/N	Relationship to woman	Sex		D.O.B			Age (yrs)	Child's birth weight (Specify in gm or lb.oz)
		M	F	DD	MM	YYYY		
1								
2								
3								
4								
5								

ADULT

S/N	Relationship to woman	Sex		Age (yrs)	Smoker (Yes=1, No=0)
		M	F		
6					
7					
8					
9					
10					

4. CHILDCARE ARRANGEMENTS

4.1. Do you have your own child or children at home under the age of 12 years?

☐ 0: No, ***go to question 5***
1: Yes

4.1.2 If yes, you are:

☐ 1: Working part time, ***go to question 4.2***
2: Working full time, ***go to question 4.2***
3: Stay home mother, ***go to question 5***

4.2. Which of the following best describes the way you arrange for your child/children aged 12 or under to be looked after while you are at work?
Please fill in numbers of relevant choices in boxes on right. You can select up to 3 choices.

1: I work only while they are at school.
2: They look after themselves until I get home.
3: I work from home.
4: My husband/partner looks after them.
5: A nanny/grandparent/relative looks after them at home
6: They go to a workplace nursery.
7: They go to a day nursery.
8: They go to a child minder.
9: A relative looks after them.
10: A friend or neighbour looks after them.
11: Others, specify_____

1st choice ☐
2nd choice ☐ ☐ No further choices
3rd choice ☐ ☐ No further choices

5. PERSONAL HEALTH

Now, I would like to ask you about your personal health and about the stress level you face.

5.1. How is your health in general? Would you say it is:

1: Very good
2: Good
3: Fair
4: Bad
5: Very bad

5.2. Do you have any long term illness or disability? By long term, I mean anything that has troubled you over a period of time**?**

0: No ***go to question 5.4***
1: Yes

5.3. What is the illness/disability? _____

_____

(Do not record headaches, indigestion, aches and pains. We are interested in major problems such as diabetes, multiple sclerosis, rheumatoid arthritis, muscular dystrophy – anything which might affect growth or body composition.)

5.4. To what extent do you feel that the stress or pressure you have experience in your life has affected your health?

1: None
2: Slightly
3: Moderately
4: Quite a lot
5: Extremely

5.5. In general, how much stress or pressure have you experienced in your daily living in the last 4 weeks?

1: None
2: Just a little
3: A good bit
4: Quite a lot
5: A great deal

5.6. Were you part of a multiple birth (twins, triplets etc.)?

0: No
1: Yes

5.8. Were you born early, late or when your maternal mother was expecting you?

1: Early
2: When expected***, go to question 5.9***
3: Late
99: Don't know***, go to question 5.9***

5.8. How early/late were you?

Wks ☐ Days ☐ 99: Don't know

5.9. How many children did your mother have before you were born? (including stillbirths)

99: Don't know

5.10. Approximately what was your weight before this pregnancy?

kg ☐ 99: Don't know

6. ASTHMA

6.1. Have you ever suffered from asthma, either as a child or an adult?

☐ 0: No ***go to question 6.3***
1: Yes
99: Don't know ***go to question 6.3***

6.1.1. If yes, was this confirmed by a doctor?

☐ 0: No
1: Yes
99: Don't know

6.2. How many attacks of wheezing have you had in the last 12 months?

☐ 0: None
1: 1-3
2: 4-12
3: More than 12

6.3. Did you suffer from eczema (recurrent itchy skin) in childhood?

☐ 0: No ***go to question 6.5***
1: Yes
99: Don't know

6.4. Have you had eczema (recurrent itchy skin) affecting the creases of your elbows or knees in the last year?

☐ 0: No
1: Yes

6.5. Have you ever had a problem with sneezing, or a runny, or blocked nose when you did not have a cold or flu?

☐ 0: No ***go to question 6.7***
1: Yes
99: Don't know ***go to question 6.7***

6.5.1. If "YES", is the nose problem usually accompanied by itchy-watery eyes?

☐ 0: No
1: Yes
2: Sometimes
99: Don't know

6.6. In the last 12 months, have you had a problem with sneezing, or a runny, or blocked nose when you did not have a cold or the flu?

☐ 0: No
1: Yes

6.7. In the last 12 months, have you used any medicines to treat hay fever, rhinitis, or any other nasal problems, at any time (including sprays, solutions, pills, capsules or tablets)?

☐ 0: No
1: Yes

7. HIGH BLOOD PRESSURE (HYPERTENSION)

7.1. Has a doctor, a nurse or other healthcare professional ever told you that you have high blood pressure?

☐ 0: No ***go to question 8.1***
1: Yes

7.2. At what age were you diagnosed to have high blood pressure? *(Fill in one of the options below)*

Age ☐☐ (or) Year ☐☐☐☐ ☐ 99: Don't know

8. DIABETES MELLITUS

8.1. Has a doctor ever told you that you have diabetes?

☐ 0: No ***go to question 9.1***
1: Yes

8.2. How old were you when the doctor first told you that you had diabetes? *(Fill in one of the options below)*

Age ☐☐ (or) Year ☐☐☐☐ ☐ 99: Don't know

9. MYOPIA

9.1. Have you ever been told by a doctor or an optometrist that you need to wear glasses or contact lenses?

☐ 0: No ***go to question 10.1***
1: Yes

9.2. Did you get the glasses / contact lenses?

☐ 0: No ***go to question 10.1***
1: Yes

9.3. When did you first begin wearing glasses or contact lenses?

Age ☐☐ (or) Year ☐☐☐☐ ☐ 99: Don't know

9.4. What is the purpose for the glasses / contact lenses?

☐ 1: Seeing far ± Astigmatism
2: Seeing near ± Astigmatism
3: Seeing both far and near
4: Astigmatism only
99: Don't know

10. FAMILY HISTORY

10.1. Do you have a history of one of the following diseases in your first degree biological relatives (immediate family members)?

☐ 0: No, ***go to question 11***
1: Yes, ***specify in the following table.***

Code	First degree relatives
1:	Father
2:	Mother
10-19:	Sisters
20-29:	Brothers
30-39:	Sons
40-49:	Daughters

Code	Site of cancer
1:	Breast
2:	Ovarian
3:	Colorectal
4:	Others, specify _____ _____
99:	Don't know

Please use multiple rows if multiple diseases per individual
Pre-eclampsia = high blood pressure in pregnancy
Code Yes=1, No=0, Don't know=99 and N.A. for Not Applicable

First degree relative	Cancer		High blood pressure	Diabetes mellitus	Myopia	Cardio-vascular disease	Pre-eclampsia
	Yes=1 No=0 Don't know =99	Site	Yes=1 No=0 Don't know=99	Yes=1 No=0 Don't know=99	Yes=1 No=0 Don't know=99	Yes=1 No=0 Don't know=99	Yes=1 No=0 Don't know=99

11. MENSTRUAL CYCLES AND PREGNANCIES

11.1. Is your usual cycle regular, or has it varied by more than 5 days between periods in the last 6 months?

☐ 1: Regular ***go to question 11.2***
2: Varied by more than 5 days ***go to question 11.3***
3: Don't know ***go to question 11.3***

11.2. How long is your usual menstrual cycle between the start of one period and the start of the next period?

☐☐ days ☐ 99: Don't know

11.3. How old were you when you had your first period?

☐☐ years ☐ 99: Don't know

11.4. **IF THIS IS YOUR FIRST PREGNANCY, PLEASE GO TO QUESTION 12.1**

Next, would you please tell me the ending date(s) and outcome(s) of each of your pregnancy in sequence?

1: Live birth – Normal vaginal delivery
2: Live birth – Assisted delivery (Forceps/vacuum)
3: Live birth – Caesarean section
4: Abortion
5: Miscarriage
6: Stillbirth
7: Premature birth – Normal vaginal delivery
8: Premature birth – Assisted delivery
9: Premature birth – Caesarean section
10: Ectopic pregnancies
11: Others, please specify:
_____

S/N	Pregnancy outcome	Year of start of pregnancy	Total weeks of pregnancy	Baby's weight (Specify in gm or in lb.oz)	If live birth, breastfed or not?		If breastfed, how long?			Pregnancy related complications						
										Hypertension		Diabetes Mellitus		Anaemia		Others (Please specify)
					0: No	1: Yes	Year(s)	Mth(s)	Wk(s)	0: No	1: Yes	0: No	1: Yes	0: No	1: Yes	
1																
2																
3																
4																
5																

11.5. Were you anaemic after the birth of any of your previous babies?

☐ 0: No
1: Yes
99: Don't know

12. MEDICATION

The questions below ask about REGULAR consumption of medications, supplements and traditional medicine in the past year BEFORE THIS PREGNANCY.

Regular refers to more than once a week for at least 1 month in past1 year.

12.1. Have you been taking any medications regularly before this pregnancy?

☐ 0: No ***go to question 12.2***
1: Yes, please specify in table below

S/N	Name of Medication
1	
2	
3	
4	
5	

12.2. Have you been taking folic acid supplement before your current pregnancy?

☐ 0: No ***go to question 12.3***
1: Yes

12.2.1. How many weeks before pregnancy have you been taking folic acid supplement?

☐☐ weeks

12.3. Are you still taking folic acid supplement **NOW**?

☐ 0: No
1: Yes

12.4. Have you been taking any fortified milk supplement (e.g. Anlene, Anmum) regularly before this pregnancy?

☐ 0: No
1: Yes

12.5. Have you been taking any probiotics (e.g. Yakult, Vitagen, Yoghurt) regularly before this pregnancy?

☐ 0: No
1: Yes

12.6. Have you been taking any other vitamins or supplements regularly before this pregnancy?

☐ 0: No
1: Yes

12.7. Have you been taking any traditional medicines regularly before this pregnancy?

☐ 0: No
1: Yes

13. INCOME

13.1. What is your personal monthly income?

☐ 1: $0 - $999
2: $1000 - $1999
3: $2000 - $3999
4: $4000 - $5999
5: more than $6000
6: Refuse to answer
99: Don't know

13.2. What is the monthly income of your household?

☐ 1: $0 - $999
2: $1000 - $1999
3: $2000 - $3999
4: $4000 - $5999
5: more than $6000
6: Refuse to answer
99: Don't know

Interview end time: _____

THANK YOU VERY MUCH FOR YOUR HELP.

Week 26-28 clinic visit
Interviewer-administered questionnaire
(Mother)

Study ID: _____ Date of interview: _____

Interviewer code: _____ Interview start time: _____

Have you changed your address or telephone number since you were seen in early pregnancy?

☐ 1.9. No

1.10. Yes: Please specify_____

Address:

Block/House no./Building Name/Street:

_____

Unit no:_____ Postal Code: _____

Week 26-28 clinic visit
Interviewer-administered questionnaire
(Mother)

1. OCCUPATIONAL ACTIVITY

1.1. Have you had any jobs at any time since you became pregnant?

☐ 0: No *go to Section 2*

1: Yes

(1) Would you please tell me your jobs during pregnancy and the weeks of your pregnancy in which you have done them?

If started before pregnancy, week started =

0 If job is still ongoing, week finished = 88

Occupation	Week started	Week finished
1.		
2.		
3		
4.		

1.3. How many hours in total did you work during an average week?

☐☐☐.☐ hrs *(round to nearest 0.5 hr)*

1.4.Did this include working night shifts?

Night shift means "working at least once a week or more from 12 midnight to 6:00am"

☐ 0: No

1: Yes

1.6. At around this time, did your paid work involve any of the following activities in an average day at work?

i) Standing or walking for more than **four** hours in total?

☐ 0: No

1: Yes

ii) Kneeling or squatting for more than an hour in total?

No

Yes

iii) Standing or sitting with your trunk bent forward for more than an hour in total?

a. No

b. Yes

☐

iv) Lifting or carrying weight of 25kg (56lbs) or more by hand (equivalent to a sack of potatoes, a nine year old child, a very heavy suitcase)

☐ a. No
b. Yes

1.6. Have you at any time during your pregnancy left a job or changed the type of work that you were doing because of a health problem?

☐ 0: No
1: Yes

1.6a. If yes, give details of health problems_____
_____

1.6b. and the stage of pregnancy ☐☐ weeks

2. ACTIVITY AND EXERCISE– BEFORE THIS PREGNANCY

Now I'm going to ask you about your activity and exercise patterns during the 1 year before your pregnancy. We would like you to divide up a "typical" day into three types of activities. These are:
(1) sleeping or lying, (2) sitting, (3) standing or walking.

2.1. Over a typical 24 hour day, how many hours do you generally spend sleeping or lying with your feet up?
(ask what time she usually goes to bed & wakes up, including any at work!)

☐☐.☐ hrs *(round to nearest 0.5 hr)*

2.2. How many hours on a typical day do you spend sitting down?
(e.g. includes sitting at work, mealtimes, driving, reading, watching TV)

☐☐.☐ hrs *(round to nearest 0.5 hr)*

2.3. This would mean that you spend about xx hours a day on your feet.
Does this sound about right?

☐☐.☐ hrs *(round to nearest 0.5 hr)*

Sum of hours reported in Q2.1, 2.2 and 2.3 should total up to 24 hours

Total hours: _____
Checked and signed: _____

2.4. Out of these xx hours spent on your feet, about how much of the time are you actively on the move (rather than standing fairly still)?

☐	1: Very little	10%
	2: Some	30%
	3: About half	50%
	4: Most	70%
	5: Almost all	90%

2.5. During the 1 year before your pregnancy, how often have you done the following kind of exercises or activities?

a) **Strenuous exercise** which normally makes your heart beats rapidly AND leaves you breathless e.g. jogging, vigorous swimming or cycling, aerobics

☐ 5.2. Never
5.3. Once every 2-3 months
5.4. Once a month
5.5. Once a fortnight
5.6. 1-2 times per week
5.7. 3-6 times per week
5.8. Once a day
5.9. More than once a day

and **on average** about how long does each period of activity last?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

b) **Moderate exercise** which normally leaves you exhausted but not breathless, e.g. brisk walking, dancing, easy swimming or cycling, badminton, sailing.

☐ 1: Never
2: Once every 2-3 months
3: Once a month
4: Once a fortnight
5: 1-2 times per week
6: 3-6 times per week
7: Once a day
8: More than once a day

and **on average** about how long does each period of activity last?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

c) **Gentle exercise** which normally leaves you tired but not exhausted, e.g. walking, driving, housework (including washing windows and polishing), gardening, DIY, golf.

☐ 1: Never
2: Once every 2-3 months
3: Once a month
4: Once a fortnight
5: 1-2 times per week
6: 3-6 times per week
7: Once a day
8: More than once a day

and **on average** about how long does each period of activity last?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

2.6.On a typical day, how many hours do you generally spend watching television?

☐ 1: More than 5 hours
2: 4-5 hours
3: 3-4 hours
4: 2-3 hours
5: 1-2 hours
6: Less than one hour
7: None

4.3. Which of the following best describes your walking speed?

☐ Very slow
Stroll at an easy pace
Normal speed
Fairly brisk
Fast

3. ACTIVITY AND EXERCISE –DURING THIS PREGNACY

Can I now ask you about your activity and exercise patterns over the last 6 months?
As before, we would like you to divide up a "typical" day into three types of activities.
These are:
(1) sleeping or lying, (2) sitting, (3) standing or walking.

3.1. Over a typical 24 hour day, how many hours do you generally spend sleeping or lying with your feet up?
(ask what time she usually goes to bed & wakes up, including any at work!)

☐☐.☐ hrs *(round to nearest 0.5 hr)*

3.2. How many on a typical day do you spend sitting down?
(e.g. includes sitting at work, mealtimes, driving, reading, watching TV)

☐☐.☐ hrs *(round to nearest 0.5 hr)*

3.3. This would mean that you spend about xx hours a day on your feet.
Does this sound about right?

☐☐.☐ hrs *(round to nearest 0.5 hr)*

Sum of hours reported in Q3.1, 3.2 and 3.3 should total up to 24 hours

Total hours: _____
Checked and signed: _____

3.4. Out of these xx hours spent on your feet, about how much of the time are you actively on the move (rather than standing fairly still)?

☐ 1: Very little 10%
2: Some 30%
3: About half 50%

4: Most	70%
5: Almost all	90%

3.5. During the past six months, how often have you done the following kinds of exercise or activities?

a) **Strenuous exercise** which normally makes your heart beat rapidly AND leaves you breathless e.g. jogging, vigorous swimming or cycling, aerobics

☐ 2 Never
3 Once every 2-3 months
4 Once a month
5 Once a fortnight
6 1-2 times per week
7 3-6 times per week
8 Once a day
9 More than once a day

and **on average** about how long does each period of activity last?

☐☐.☐ hrs *(round to nearest 0.5 hr)*

b) **Moderate exercise** which normally leaves you exhausted but not breathless, e.g. brisk walking, dancing, easy swimming or cycling, badminton, sailing.

☐ 1: Never
2: Once every 2-3 months
3: Once a month
4: Once a fortnight
5: 1-2 times per week
6: 3-6 times per week
7: Once a day
8: More than once a day

and **on average** about how long does each period of activity last?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

c) **Gentle exercise** which normally leaves you tired but not exhausted, e.g. walking, driving, housework (including washing windows and polishing), gardening, DIY, golf.

☐ 1: Never
2: Once every 2-3 months
3: Once a month
4: Once a fortnight
5: 1-2 times per week
6: 3-6 times per week
7: Once a day
8: More than once a day

and **on average** about how long does each period of activity last?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

3.6.On a typical day, how many hours do you generally spend watching television?

☐ 1: More than 5 hours
2: 4-5 hours
3: 3-4 hours
4: 2-3 hours
5: 1-2 hours
6: Less than one hour
7: None

3.7. Which of the following best describes your walking speed?

☐ 1: Very slow
2: Stroll at an easy pace
3: Normal speed
4: Fairly brisk
5: Fast

4. CONTRACEPTION

4.1.How many weeks pregnant were you when you first found out that you were pregnant? wks ☐☐

4.2.Was this pregnancy planned?

☐ 0: No *Go to question 4.4*
1: Yes: *Go to question 4.3*

4.4 If YES, did you change your diet when you were planning to be pregnant?

☐ 0: No *Go to question 5.1*
1: Yes *Go to question 5.1*

4.4.If NO, this pregnancy is due to

☐ 2 No contraception: *Go to question 5.1*
3 Failure of contraceptive methods

4.5.If NO, which was the main contraceptive method used which failed?

☐ 1. Safe period
2. Barrier e.g. condom, diaphragm
Hormones
3.a. Pills
3.b. Patch
3.c. Injection
3.d. Implants
4. Intrauterine contraceptive device
5. Withdrawal
6. Others: specify _____

5. DIET DURING PREGNANCY

5.9.Are you following any special diet?

☐ 1 No *go to question 5.3*
2 Yes

5.10. If yes, what is your special diet?

☐ 99: Vegetarian (Eggs and milk allowed)
100: Vegan (No eggs or milk allowed) 3. Diabetic diet
4. Low fat diet
5. Others, specify _____

(1) How often do you eat eggs?

☐ More than one egg a day
One egg a day
3. 4 to 6 eggs a week
4. 1 to 3 eggs a week
5. Less than one egg a week
6. Do not eat eggs at all

5.4 How often do you eat liver (any type e.g. chicken, beef, pork)?

☐ 1: Every day
2: 4 to 6 times a week
3. 1 to 3 times a week
4. Less than once a week but more than once a month
5. Less than once a month
6. Do not eat liver at all

5.5 How often do you eat out or purchase take-away foods?

☐ 1: Two meals a day or more
2: One meal a day
3. 4 to 6 meals a week
4. 1 to 3 meals a week
5. Less than once a week
6. Never/ rarely

2 I would like to find out more about your diet during pregnancy compared to what you usually ate before you were pregnant. I will be asking you about your eating habit for a list of foods during pregnancy. Please tell me if you ate more, less or similar amount of the food during pregnancy compared to your usual diet.

	Types of Food	**Change in amount**
1.	Chicken	
2.	Fish	
3.	Meat (beef / mutton / pork)	
4.	Organ meats (e.g. liver, kidney, heart, brain)	
5.	Seafood (e.g. prawn, crab, mussels, clams)	
6.	Egg	
7.	Vegetables (all types)	
8.	Fruits (all types)	
9.	Red, orange, yellow fruits and vegetables (e.g. carrots, papaya)	
10.	Rice, noodles, breads	
11.	Cheese, yogurt	
12.	Chocolates, sweets, biscuits, cakes	
13.	Milk	
14.	Chocolate drinks (Milo, Ovaltine)	
15.	Soft drinks (e.g. Coke, sprite, 7-up, Pepsi)	
16.	Tea	
17.	Coffee	
18.	Wine/alcohol (including tonic wine)	

Key
1: More
2: Less
3. Same as before
9. Don't usually eat

6. APPETITE AND NAUSEA DURING PREGNANCY

6.1. Have you experienced any nausea or sickness since becoming pregnant?
0: No *go to question 6.5*
1: Yes

6.2. If yes, has this been:
1: Mild (nausea only)
2: Moderate (sometimes sick, vomiting)
3: Severe (regularly sick, vomiting, can't retain meals)

6.3. If yes, were you admitted to the hospital because of nausea?
0: No *go to question 6.5*
1: Yes

☐

6.4. If yes, how were you treated?

☐ 1: Fasting, then slowly introducing food
2: Intravenous fluid treatment
3: Medication *(Note: Refer to medical records/CPSS)*

6.5. **Compared with BEFORE you were pregnant**, are you eating:

☐ 1: More *go to question 6.5a*
2: The same *go to question 7.1*
3: Less in amount *go to question 6.5b*
99: Don't know

6.5a. If **more**, is this:

☐ 6.8. Because you feel more hungry
6.9. To prevent from feeling sick
6.10. Because you feel it is best for the baby
6.11. Other reasons; specify: _____

6.5b. If **less**, is this:

☐ 1: Because you feel less hungry
2: Because of nausea/sickness
3: Don't want to put on too much weight
4: Other reasons; specify: _____

2 DIETING

7.3. Which of the following describes you best?

☐ 1 I have NEVER been on a diet to lose weight.
2 I have ONLY ONCE been on a diet to lose weight.
3 I USED TO diet REGULARLY to lose weight but NOT ANYMORE
4 I go on a diet to lose weight EVERY NOW AND AGAIN.
5 I am USUALLY on a diet to lose weight.

If answered 2, 4, 5, please ask question 7.2; otherwise go to next section.

2 Are you currently trying to lose weight by dieting?

☐ 1: No
2: Yes

8. ALCOHOL CONSUMPTION – BEFORE THIS PREGNANCY

I'd like to ask you a few questions about your drinking and smoking habits.

8.1 Did you ever drink alcohol before this pregnancy?

☐ 0: No *go to section 9*
1: Yes
1 Don't know

9.2. How often did you drink the following alcoholic beverages in the 1 year before you became pregnant? Please select the category that best describes how often and how much you drank during the past year.

Alcoholic beverages	Average consumption in past year	Usual serving size
Beer	☐ 1. Never or hardly ever 2. Once a month 3. 2-3 times a month 4. Once a week 5. 2-3 times a week 6. 4-6 times a week 7. Once a day 8. 2 or more times a day	☐ 1. One small bottle (375ml) or less 2. One large bottle (750ml) 3. Two large bottles 4. Three large bottles or more
Wine (eg. red wine)	☐	☐ 1. One wine glass (118ml) or less 2. Two wine glasses 3. Three wine glasses 4. Four wine glasses or more
Traditional wine (eg. DOM)	☐	☐ 1. One wine cup (30ml) or less 2. Two wine cups 3. Three wine cups 4. Four wine cups or more
Hard liquor (eg. brandy)	☐	☐ 1. One drink (30ml) or less 2. Two drinks 3. Three drinks 4. Four drinks or more

9 ALCOHOL CONSUMPTION – DURING THIS PREGNANCY

Did you ever drink alcohol during this pregnancy?

☐ 0: No *go to section 10*
1: Yes
2: Refuse to answer

9.1 During the past 6 months, how often did you drink the following alcoholic beverages? Please select the category that best describes how often and how much you drank.

Alcoholic beverages	Average consumption past 6 mth	Usual serving size
Beer	☐ 1. Never or hardly ever 2. Once a month 3. 2-3 times a month 4. Once a week 5. 2-3 times a week 6. 4-6 times a week 7. Once a day 8. 2 or more times a day	☐ 1. One small bottle (375ml) or less 2. One large bottle (750ml) 3. Two large bottles 4. Three large bottles or more
Wine (eg. red wine)	☐	☐ 1. One wine glass (118ml) or less 2. Two wine glasses 3. Three wine glasses 4. Four wine glasses or more
Traditional wine (eg. DOM)	☐	☐ 1. One wine cup (30ml) or less 2. Two wine cups 3. Three wine cups 4. Four wine cups or more
Hard liquor (eg. brandy)	☐	☐ 1. One drink (30ml) or less 2. Two drinks 3. Three drinks 4. Four drinks or more

10. PERSONAL VIEWS ON BREAST FEEDING

10.1 Have you breastfed before?

☐ 0: No, *go to question 10.3*
1: Yes

10.1.1 If "YES", how many children have you breastfed before?

☐☐ Number of children

10.1.2 If YES, please describe your type of breastfeeding for your last child:

(1) Exclusive breastfed (Only breast milk with no water)

☐ (2) Predominant breastfed (Breast milk and liquids (including water) other than formula)

(3) Partial breastfed (Breast milk, formula and liquids)

10.1.3 How long did you breastfeed your last child?

☐ Year ☐☐ Months ☐☐ weeks

10.2 Are you still breastfeeding during this pregnancy?

☐ 0: No

1: Yes but I stopped at ☐☐ weeks of pregnancy

Yes, I am still continuing breastfeeding

10.3 Do you know people who have successfully breastfed their babies?

☐ 0: No

1: Yes

10.4 Did you receive advice from family or friends about breastfeeding?

☐ 0: No

1: Yes

10.5 Have you read books or watched programs on breastfeeding?

☐ 0: No

1: Yes

10.6 Are you currently attending antenatal classes?

☐ 0: No

1: Yes

10.7 Do you plan to breastfeed?

☐ 0: No

1: Yes, for how long ☐☐ months , *go to question 10.8*

Don't know

10.7.1 If No, please specify reason

☐ 1: Underlying medical problems

2: Painful

3: Troublesome

4: Inconvenient

5: Formula more nutritious

6: No reason

7: Others, specify _____

11.3.Who will be the main person helping you with the baby after delivery?

☐ Confinement nanny
Mother / Mother-in-law
Husband
Other relatives
Others, specify_____

11. SMOKING – BEFORE THIS PREGNANCY

11.1 Have you ever smoked regularly (at least once a day for a year or more)? ☐
0: No *go to question 11.5*
1: Yes
2: Refuse to answer

11.2 How old were you when you first smoked regularly?

☐☐ yrs

11.3 Did you smoke during the 1 year before you became pregnant? ☐
0: No *go to question 11.5*
2: Yes

(1) If yes, how many sticks per day? *Record maximum stated.*

☐☐

Note to interviewer: You may want to explain to the participant that even though she does not smoke, there is some evidence of health implications from second-hand smoke exposure. The following questions are to capture information on second-hand smoke exposure, i.e. where the participant was close enough to the smoker(s) to smell the smoke.

11.5 Did anyone living in your home smoke at home on a daily basis for 6 months or longer? ☐
0: No *go to question 11.7*
1: Yes

11.6 For how many years did at least 1 person living in your home smoke daily at home? ☐
1: 1 year or less
2: 2-5 years
3: 5-14 years
4: 15-24 years
5. 25+ years

11.7 Have you ever had a job in which, on a daily basis, you were exposed to cigarette smoke from others?
0: No
1: Yes

12. SMOKING – DURING THIS PREGNANCY

12.1 Are you currently smoking?

☐ 0: No *go to question 12.3*
7: Yes

2 If yes, how many sticks per day? *Record maximum stated.*

☐☐

3 During your pregnancy, did anyone living in your home smoke at home on a daily basis?

☐ 99: No
100: Yes

4 During your pregnancy, have you ever had a job in which, on a daily basis, you were exposed to cigarette smoke from others?

☐ 0: No *go to section 13*
7: Yes

(1) On average, how many hours were you exposed to cigarette smoke at work?

☐ 1 hour or less
1-3 hours
More than 3 hours

(2) Are you currently exposed to cigarette smoke at work on a daily basis?

☐ 0: No
1: Yes

13. MEDICATION

13.1 Are you taking any medications / supplements / traditional medicine regularly DURING this pregnancy?

Regular refers to more than once a week

☐ 0: No *END OF QUESTIONNAIRE*
1: Yes, please specify in table below

S/N	Name of Medication
1	
2	
3	
4	
5	
S/N	**Name of Supplement**
1	
2	
3	
4	
5	
S/N	**Name of Traditional Medicine**
1	
2	
3	
4	
5	

THANK YOU VERY MUCH FOR YOUR HELP!

Interview end time: _____

GUSTO
GROWING UP IN SINGAPORE TOWARDS HEALTHY OUTCOMES

26-28 Week clinic visit
Mother's Case Report Form

Study ID: _____ Date of interview: _____

Interviewer code: _____ Interview start time: _____

		Tick when completed	Investigator code
1.	**Registration**		
2.	**26-28 wk visit questionnaires**		
2.1.	**Mother's questionnaire**		
2.2.	**Mother's self administered questionnaires**		
	☐ STAI		
	☐ EPDS		
	☐ BDI-II		
	☐ LYDON Maternal		
	☐ LYDON Domestic Helper		
3.	**Anthropometric measurements**		
3.1.	Weight		
3.2.	Height		
3.3.	Mid-upper arm circumference		
3.4.	Triceps skinfold		
3.5.	Biceps skinfold		
3.6.	Subscapular skinfold		
3.7.	Suprailiac skinfold		
4.	**Collection of hair**		
5.	**Collection of buccal swab**		
6.	**Pulse wave velocity**		
7.	**Auto Refraction**		
8.	**Fundus photography**		
9.	**Case file completed/checked**		

1.11. Anthropometric Measurements

0: Weight

			.		**kg**
			.		**kg**
			.		**kg** should be taken only if the first 2 measurements differed by >200gm.

3.2. Height

			.		**cm**
			.		**cm**
			.		**cm** should be taken only if the first 2 measurements differed by >1.0cm.

3.3. Mid-upper arm circumference

		.		**cm**
		.		**cm**
		.		**cm** should be taken only if the first 2 measurements differed by >1.0cm.

3.4. Triceps skinfold

			.		**mm**
			.		**mm**
			.		**mm**

3.5. Biceps skinfold

			.		**mm**
			.		**mm**
			.		**mm**

3.6. Subscapular skinfold

			.		
			.		**mm**
			.		**mm**
			.		**mm**

3.7. Suprailiac skinfold

			.		
			.		**mm**
			.		**mm**
			.		**mm**

Skin fold calipers used

4. Hair Collection

Taken Not taken Refused

Number of hair strands

5. Collection of buccal swab

Done Not done Refused

Number of swabs

6. Pulse wave velocity

Done Not done Refused
(Please attach report)

6 Fundus Photography:

1: **Fundus Photo**

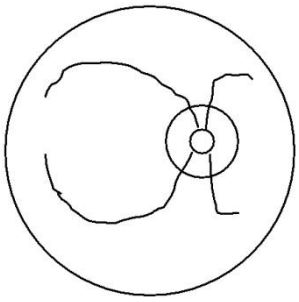
Taken Not taken Refused Unable

Comments_____

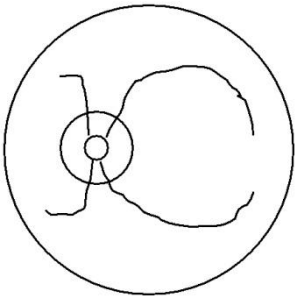
Right Eye _____

Left Eye _____

7.2. **Fundus**



(RE)



(LE)

	1. Normal 2. Abnormal 3. Unable	**1.Normal 2. Abnormal 3. Unable**
Macular [macular]		
Disc [Discr]		
Media [mediar]		
Posterior Pole of retina [postretr]		
Peripheral retina[periretr]		
Describe lesion [desbr]	_____	_____

8. Auto-Refraction(Undilated with Table-Mounted)

Taken Not taken Refused Unable

Comments_____

Right Eye _____

Left Eye _____

PLEASE WRITE THE STUDY ID AND MAKE A COPY OF THE AUTO REFRACTION REPORT AS THE DATA FADES AWAY WITHIN ONE WEEK

PLEASE PASTE THE COPIED REPORT PAGE HERE

Check the following:

2.3. Ensure best readings
2.4. Cross-out readings with *and extra readings in excess of 5
2.5. Retake if more than 1*or SD.+/- 0.25
2.6. Write down comments for any rejection or unsuccessful attempts

Right Eye _____

Left Eye _____

National University Hospital

Edinburgh Postnatal Depression Scale[1]

Date: _____ Criteria:

T1	T2	T3	PN

Sticky label

Please put a tick (✓) in the box that applies to you after each statement which comes closest to how you have felt in the PAST 7 DAYS, not just how you feel today.

1	**I have been able to laugh and see the funny side of things**
	As much as I always could
	Not quite so much now
	Definitely not so much now
	Not at all
2	**I have looked forward with enjoyment to things**
	As much as I ever did
	Rather less than I used to
	Definitely less than I used to
	Hardly at all
3	**I have blamed myself unnecessarily when things went wrong**
	Yes, most of the time
	Yes, some of the time
	No, not very often
	No, never
4	**I have felt worried and anxious for no very good reason**
	No, not at all
	Hardly ever
	Yes, sometimes

	Yes, very often	
5	**I have felt scared or panicky for no very good reason**	
	Yes, quite a lot	
	Yes, sometimes	
	No, not much	
	No, not at all	
6	**Things have been getting on top of me**	
	Yes, most of the time I haven't been able to cope at all	
	Yes, sometimes I haven't been coping as well as usual	
	No, most of the time I have coped quite well	
	No, I have been coping as well as ever	
7	**I have been so unhappy that I have had difficulty sleeping**	
	Yes, most of the time	
	Yes, sometimes	
	Not very much	
	No, not at all	
8	**I have felt sad or miserable**	
	Yes, most of the time	
	Yes, quite often	
	Not very often	
	No, not at all	
9	**I have been so unhappy that I have been crying**	
	Yes, most of the time	
	Yes, quite often	
	Only occasionally	

	No, never		
10	**The thought of harming myself has occurred to me**		
	Yes, quite often		
	Sometimes		
	Hardly ever	**Total:**	
	Never		

1 This scale has also been validated for use in antenatal women.

SELF-EVALUATION QUESTIONNAIRE **STAI Form Y-1**

Please provide the following information:

Name _____ Date _____ S

Age _____ Gender (*Circle*) **M F** T

DIRECTIONS:

A number of statements which people have used to describe themselves are given below. Read each statement and then blacken the appropriate circle to the right of the statement to indicate how you feel *right* now, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel confused	1	2	3	4
19. I feel steady	1	2	3	4
20. I feel pleasant	1	2	3	4

SELF-EVALUATION QUESTIONNAIRE STAI Form Y-2

Name_____Date_____

DIRECTIONS

A number of statements which people have used to describe themselves are given below. Read each statement and then blacken in the appropriate circle to the right of the statement to indicate you *generally* feel.

	ALMOST NEVER	SOMETIMES	OFTEN	ALMOST ALWAYS
21. I feel pleasant	1	2	3	4
22. I feel nervous and restless	1	2	3	4
23. I feel satisfied with myself	1	2	3	4
24. I wish I could be as happy as others seem to be	1	2	3	4
25. I feel like a failure	1	2	3	4
26. I feel rested	1	2	3	4
27. I am "calm, cool, and collected"	1	2	3	4
28. I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
29. I worry too much over something that really doesn't matter	1	2	3	4
30. I am happy	1	2	3	4
31. I have disturbing thoughts	1	2	3	4
32. I lack self-confidence	1	2	3	4
33. I feel secure	1	2	3	4
34. I make decisions easily	1	2	3	4
35. I feel inadequate	1	2	3	4
36. I am content	1	2	3	4
37. Some unimportant thought runs through my mind and bothers me	1	2	3	4
38. I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
39. I am a steady person	1	2	3	4
40. I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

Appendix. Pittsburgh Sleep Quality Index (PSQI)

Name _____ ID # _____ Date _____ Age _____

Instructions:

The following questions relate to your usual sleep habits during the past month *only*. Your answers should indicate the most accurate reply for the *majority* of days and nights in the past month. Please answer all questions.

1. During the past month, when have you usually gone to bed at night?

   USUAL BED TIME _____

2. During the past month, how long (in minutes) has it usually take you to fall asleep each night?

   NUMBER OF MINUTES _____

3. During the past month, when have you usually gotten up in the morning?

   USUAL GETTING UP TIME _____

4. During the past month, how many hours of *actual sleep* did you get at night? (This may be different than the number of hours you spend in bed.)

   HOURS OF SLEEP PER NIGHT _____

For each of the remaining questions, check the one best response. Please answer *all* questions.

5. During the past month, how often have you had trouble sleeping because you...

   (a) Cannot get to sleep within 30 minutes

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

   (b) Wake up in the middle of the night or early morning

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

   (c) Have to get up to use the bathroom

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

   (d) Cannot breathe comfortably

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

   (e) Cough or snore loudly

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

   (f) Feel too cold

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

   (g) Feel too hot

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

   (h) Had bad dreams

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

   (i) Have pain

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week ___

(j) Other reason(s), please describe _____

_____

How often during the past month have you had trouble sleeping because of this?

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

6. During the past month, how would you rate your sleep quality overall?

   Very good _____
   Fairly good _____
   Fairly bad _____
   Very bad _____

7. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

8. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

   No problem at all _____
   Only a very slight problem _____
   Somewhat of a problem _____
   A very big problem _____

10. Do you have a bed partner or roommate?

    No bed partner or roommate _____
    Partner/roommate in other room _____
    Partner in same room, but not same bed _____
    Partner in same bed _____

If you have a roommate or bed partner, ask him/her how often in the past month you have had...

(a) Loud snoring

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

(b) Long pauses between breaths while asleep

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

(c) Legs twitching or jerking while you sleep

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

(d) Episodes of disorientation or confusion during sleep

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

(e) Other restlessness while you sleep; please describe _____

_____

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

PUBLICATIONS